New Zealand Journal of Agricultural Research

VOLUME 2 No. 1
FEBRUARY 1959

COMMONWEALTH AGRIC. BUREAUX
18 MAR 1959

NOTICE TO CONTRIBUTORS

General

Papers published, or offered for publication, elsewhere are not acceptable. Nevertheless, publication elsewhere of an abstract or extended summary does not preclude publication in full in this journal.

Typescript

The original and one carbon copy are required, on one side only of foolscap paper, double spaced, with a left-hand margin of at least one inch and a quarter. Reasonably heavy good-quality paper should be used; flimsy paper delays the machine operator and consequently increases the cost of printing.

A brief summary is required, at the beginning of the paper. It should indicate the scope of the paper and give the principal results, and should be suitable for reproduction by abstracting journals as it stands.

All matter to be printed in italic type (e.g. generic and specific names) must be underlined.

Tables

Tabular matter costs more to print than letterpress and should therefore be kept to a minimum, bearing in mind, however, that a well planned table can take the place of a much greater area of letterpress.

Wherever possible, tables should be arranged to fit upright on the page, not sideways. They will be printed in 8-point type, and must be limited in size to fit the printed page, i.e. $4\frac{1}{4} \times 7$ in. maximum.

All tables, however brief, should be numbered, in arabic numerals. Numerous very small tables should be avoided.

Titles should be as brief as possible.

Units of measurement should be placed in parenthesis at the head of the column, not in the body of the table. Descriptive notes should be kept to the minimum, and abbreviations used wherever possible. For abbreviations, etc., the usage followed is that of British Standard 1991, Letter Symbols, Signs, and Abbreviations. Part 1. General.

Figures

These are to be numbered consecutively in arabic numerals, regardless of whether they are half-tones (photographs) or line blocks (graphs, etc.). Each must be referred to in the text, and only such figures as are essential to elucidate the text can be published. Graphs showing the same data as tables are not accepted.

The author's name, the title of the paper (abbreviated), and the figure number, should be written lightly in soft pencil on the back of each figure.

The maximum size for any figure is $4\frac{1}{4} \times 7$ in., less the space required for the caption.

Photographic prints should be on glossy paper, and may be "same size" or larger than the printed picture. Components of a composite figure should be firmly mounted on white card, and lettered as required A, B, C, etc.

Graphs should preferably be drawn about twice the final size, with the curves evenly inked throughout. Great care should be taken to ensure that all detail, especially lettering, is of such size as to be clearly legible when reduced to the final size. If he wishes, the author may insert lettering in pencil for re-draughting in this office.

If the scale is important, it should be shown on the figure, or, alternatively, the magnification or reduction may be stated in the caption, taking into account the printing reduction.

The figure number should not appear on the figure itself, only in the caption. Captions for all figures should be typed together at the end of the paper.

References

In the text, citations are to be by author's name and year, e.g. "(Brown 1957" or ". . . . as stated by Brown (1957)". The list of references at the end of the paper is to be arranged in alphabetical order of authors' surnames. Titles of papers cited may be included or omitted; if included, all words other than conjunctions and pronouns are to have capital initial letters. Titles of periodicals are to be abbreviated in accordance with the "World List of Scientific Periodicals" 3rd ed. 1952. The title of the periodical and the volume (and part) number are to be underlined for italics, but not the page number(s). Titles of books are to be in inverted commas and not underlined; the name and location of the publisher are to be given. For arrangement, punctuation, etc.. of references, see examples in this issue.

NEW ZEALAND JOURNAL
of
AGRICULTURAL RESEARCH

Department of Scientific and Industrial Research, Wellington
Editor: M. A. Black.

VOL. 2. FEBRUARY 1959 Number 1

THE INSECT COMPLEX OF SOWN PASTURES IN THE NORTH ISLAND

II. THE HEMIPTERA AS REVEALED BY SUMMER SWEEP-SAMPLING

By R. A. CUMBER, Entomology Division, Department of Scientific and Industrial Research, Palmerston North.

(*Received for publication, 6 August 1958*)

Summary

A sweep-sampling survey during the summer of 1957 revealed 49 species of Hemiptera in the 9 main pasture types found in the North Island. The identity, distribution, abundance, and possible economic importance of each species are discussed.

INTRODUCTION

During the summer of 1957, a survey of insects associated with pastures in the North Island was carried out. In an earlier paper (Cumber 1958) techniques, coverage, and relevant sample-station details are given, and the insect complex generally as shown by sweep-sampling is analysed and discussed.

The present account gives information in respect of the species of Hemiptera taken during sweep-sampling. The identity of the species, their distribution, and the numbers encountered are shown, and their possible importance in the light of systematic position and known habits is discussed.

The survey involves 113 paddock and 108 adjacent roadside samples each comprising a single sweep from an area of approximately 8 square yards.

Distribution maps for 16 species are presented, the presence of a species being indicated by a solid black circle in contrast to an open ring where sampling failed to reveal its presence.

RESULTS

HEMIPTERA – HOMOPTERA

Fam. JASSIDAE

He. 5. *Erythroneura zealandica* Myers

Distribution

Myers (1923), in describing this as a new species, states: "This is the common grass-frequenting species, found in large numbers from any grassy places, whether the grass be introduced pasturage or native tussock. It is also common on . . . tauhinu (*Cassinia leptophylla*), taken on mangrove at Whangarei . . . from manuka (*Leptospermum scoparium*) . . . Tararua Range 4,000–5,000 ft." Cottier (1931) records it from many localities in the southern portions of the South Island.

The distribution encountered in the North Island is shown in Fig. 1. It coincides with Myer's distribution of Whangarei to Wellington, and it is seen that the species is more consistently present in the southern portions. Its presence at high altitudes in the Tararua Range indicates its ability to cope with pasture conditions in southern cooler areas.

Habits

Myers reports the species as having been taken after mid-winter, indicating "it is possible that many of our species (Typhlocybinae) are carried over the winter as imagines." Cottier however records "a few adults and plenty of eggs during the winter".

This is certainly one of the more commonly encountered pasture insects in the North Island. It occurred in 52 paddock (984 specimens) and 35 roadside (234 specimens) samples. The maximum population encountered was in a paddock sample from Marton on 27.3.57 which contained 162 adults and 4 nymphs. Adults and nymphs were taken in January, February, and March.

Economic Significance

This species could be of very great importance in pastures, but considerable work would be necessary to prove it. Damage could be caused through mechanical means, toxin injection, or transmission of virus diseases.

The insect is present in considerable numbers. Chlorotic spotting of leaves has been noted by the writer. It is possible that some of the viruses affecting our grasses and clovers are transmitted by this species.

He. 11. *Xestocephalus* sp.

Distribution

The genus is world-wide. Wolcott (1937) records high populations in northern New York State. Tillyard (1926) records it as being common to Australia and New Zealand. The distribution of this species is shown in Fig. 2, where it is seen to occur from the Bay of Plenty southwards.

Habits

These are unknown, as is the identity of the species. It was taken in 6 paddock (37 specimens) and 7 roadside (12 specimens) samples, the maximum catch being 28 adults in the case of a paddock sample

Fig. 1.—Distribution map for the jassid *Erythroneura zealandica*. He. 5.
Fig. 2.—Distribution map for the jassid *Xestocephalus* sp. He. 11.

from Taueru on 25.3.57. Adults were taken in January, February, and March. No nymphs were taken.

Economic Significance

No very high populations were encountered, so at present it would appear that any economic importance is likely to be related to its capacity to transmit virus diseases. It may be shown eventually however, that it is associated with a relatively sparse weed.

He 13. *Thamnotettix* sp.

Distribution

The genus is of world-wide distribution. Quinton (1956) records it from forage crops in Connecticut. Osborn (1939) also refers to pasture- and cereal-damaging species in North America. The distribution of this species is shown in Fig. 3, where it is seen to occur widely in the North Island although it was not taken north of Auckland.

Habits

The species occurred in 19 paddock (249 specimens) and 6 roadside (26 specimens) samples, the maximum number taken being in the case of a paddock sample from Waitoa on 5.3.57 which yielded 23 adults and 88 nymphs. Nymphs are recorded for February and March, and adults for January, February, and March, indicating that the species probably overwinters as adults.

Fig. 3.—Distribution map for the jassid *Thamnotettix* sp. He. 13.
Fig. 4.—Distribution map for the jassid *Thamnotettix* sp. He. 14.

Economic Significance

There is no detailed information on host plants, habits, etc., so that the species is only under suspicion. Populations are considerable, however, and its systematic position makes it a possible virus vector and a species which could cause considerable mechanical or toxic damage under build-up conditions.

He. 14. *Thamnotettix* sp.

Distribution

The same general remarks apply as in species He. 13. The distribution of this species is shown in Fig. 4, which indicates that it occurs throughout the North Island.

Habits

This species was taken in 21 paddock (66 specimens) and 3 roadside (3 specimens) samples, the maximum number being from a paddock sample at Tutira on 11.2.57 which contained 27 adults. Adults were taken in January, February, and March, and nymphs in February and March.

Economic Significance

Host associations are not known, but virus transmission and mechanical and toxin damage are possibilities.

He. 15.

It has not been possible to place this species in a known genus. It was taken from roadside and paddock near Wallingford on 26.3.57 (4 specimens). It is of no economic importance and is probably associated with a pasture weed.

He. 19. *Deltocephalus* sp.

Distribution

The genus is of world-wide distribution. This species is widely distributed in the North Island as shown by Fig. 5, but did not appear in samples north of Auckland.

Habits

No information on this species is available. It was taken in 20 paddock (197 specimens) and 5 roadside (5 specimens) samples, the maximum catch of 40 adults being in a paddock sample from Greytown on 25.3.57. Nymphs and adults were taken in January, February, and March.

Economic Significance

This genus contains serious pasture pests (Quinton 1956; Osborn 1939), and the high populations met indicate that the species should be regarded with considerable suspicion. There is the possibility of mechanical damage, toxins, or virus transmission.

He. 32. *Edwardsiana australis* (Froggatt) (Australian Apple Leaf-hopper).

Myers (1923) notes this species as occurring in the Auckland, Hawke's Bay, and Nelson orchard areas where it is damaging to the foliage of apple and hawthorn. Atkinson *et al.* (1956) records it as "General throughout apple-growing areas of the country".

A single adult was taken at Wairoa on 12.2.57. The species is not associated with pastures.

Fam. DELPHACIDAE

He. 4. *Delphacodes* sp.

Distribution

The genus is world-wide occurring in both Australia and New Zealand. This species was taken at Horotiu (23.1.57), Maraekakaho (11.2.57), Napier (11.2.57), Wairoa (12.2.57), Gisborne (13.2.57), Te Matai (4.3.57), and Mangaweka (18.3.57), indicating a general distribution.

Habits

It was taken in 6 paddock (20 specimens) and 4 roadside (10 specimens) samples, the maximum number being 12 adults and 1 nymph in the paddock sample from Te Matai. Adults were taken in

Fig. 5.—Distribution map for the jassid *Deltocephalus* sp. He. 19.
Fig. 6.—Distribution map for the delphacid *Delphacodes tasmani*. He. 7.

January, February, and March, and a single nymph in February. No further information is available.

Economic Significance

Not considered important.

He. 7. *Delphacodes tasmani* Muir

Distribution

This species was described in 1923 from material taken at Waikanae and in the Tararua Range.

The distribution encountered is shown in Fig. 6, which indicates that the species is widespread throughout the North Island.

Habits

Muir gives no information on host plants. In the present survey the species was taken in 15 paddock (29 specimens) and 7 roadside (8 specimens) samples, the maximum population being 9 adults from a paddock sample at Kohinui on 28.3.57. Adults were also taken in January and February.

Economic Significance

This species is not considered to be of importance.

He. 20. *Nilaparvata myersi* Muir

Distribution

This species was described in 1923 from a single male taken on Mt. Alpha, Tararua Range, at 4,500 ft. In the present survey 2 paddock and 1 roadside samples each yielded a single adult specimen, the localities being Lake Tutira (11.2.57), Gisborne-Tiniroto (12.2.57), and Waikohu (13.2.57).

Habits and Economic Significance

The habits of this species, which is of no economic importance, are not known.

He. 48. *Delphacodes* sp.

A single male was taken in a roadside sample from Wairoa on 5.3.57. No information is available on this species.

He. 49. *Delphacodes* sp.

A single specimen was taken in a paddock sample from Helensville on 23.1.57. No information is available on this species.

Fam. DICTYOPHARIDAE

He. 21. *Thanatodictya tillyardi* Myers

Distribution

This species was described in 1923 from specimens taken on the Dun Mountain, Nelson, at 3,000 ft. In the present survey it was taken at Whangaroa (17.1.57), Maraekakaho (11.2.57), Rotorua (4.3.57), and Hikateruru (6.3.57), the greatest number taken being 3 in the Whangaroa sample.

Habits

The writer has noticed a close association between this species and bracken fern (*Pteridium esculentum* Hook.) in the far northern areas. This association was also recorded by Myers. However no fern was noted at the above localities with the exception of Whangaroa.

Economic Significance

The species is of no importance in pastures.

Fam. CIXIIDAE

He. 2. *Oliarus oppositus* (Walker)

Distribution

Myers (1924) indicates that this species is "widespread over both Islands and to an elevation of at least 4,500 ft (Tararua Range)". The writer has collected it from Kaitaia to Wellington (Cumber 1953a) but knows of no records from east of the main ranges running from Wellington to the East Cape. The distribution as indicated by the survey is shown in Fig. 7.

Habits

Myers states that "this species frequents grass and ground herbage". It is probable that the life history closely follows that of the allied species *Oliarus atkinsoni* Myers which has been studied in detail (Cumber 1952a, b). In *O. atkinsoni* there is one generation each year, the adults appearing in spring from nymphs which feed on surface or sub-surface rootlets in small galleries. For this reason, only adults may be taken by sweeping. It appeared in 4 paddock (4 specimens) and 11 roadside (34 specimens) samples, indicating its preference for the longer herbage and relatively undisturbed conditions. The maximum catch was from a roadside sample at Whangaehu on 30.1.57 which yielded 14 adults. Adults are known to occur from November to March.

Fig. 7.—Distribution map for the cixiid *Oliarus oppositus*. He. 2.
Fig. 8.—Distribution map for the aphid *Rhopalosiphum splendens*. He. 38.

Fam. FLATIDAE

He. 35. *Sephena cinerea* Kirk.

Distribution

Myers (1922) describes this species as "an introduced Australian species which carries fireblight." It is widespread throughout the North Island and the north of the South Island. It was taken in the present survey at Levin (31.1.57), Gisborne-Tiniroto (12.2.57) and Taumaranui (8.3.57). Only 5 adults were taken.

Habits

These have not been studied in New Zealand.

Economic Significance

This species is an important pest of shrubs and garden plants, but is not important in pastures.

Fam. RICANIIDAE

He. 28. *Scolypopa australis* Walk. (Passion-vine Hopper)

Distribution

This is regarded as an Australian species which has been in the country at least since 1897 (Hutton 1897). It is now widespread in the North Island and the north of the South Island. In the present survey single adults were taken at Uruti (24.1.57), Tutira (11.2.57), Te Matai (4.3.57), and Tauranga (5.3.57).

Habits

This species has not been studied in detail in New Zealand. In Australia it does not occur in dense populations comparable with those met in New Zealand on native vegetation and in gardens and orchards. This suggests that it is controlled in the former country by parasites or predators which do not exist here.

Economic Significance

The species is undoubtedly damaging to trees, shrubs, and garden plants, but is not of importance in pastures. Paterson (1947) incriminated this species in connection with poison honey through its production of contaminated honey-dew when feeding on *Coriaria arborea* Lindsay in central North Island districts.

Fam. PSYLLIDAE

He. 31. *Ctenarytaina pollicaris* Ferris et Klyver

Tuthill (1952) indicates that this species is present in both North and South Islands, its host plant being *Leptospermum ericoides* A. Rich. In the present survey single adults were taken at Kaitaia (16.1.57), Whangaroa (17.1.57), and Wellsford (22.1.57), where adjacent *Leptospermum* was undoubtedly responsible for its presence. It is restricted to its host and is therefore of no importance in pastures.

Fam. APHIDIDAE

He. 8. *Hypermyzus lactucae* (L.)

Distribution

This species is of world-wide distribution. Cottier (1953) records it from Auckland and Cape Terawhiti. In the present survey it was taken at Warkworth (22.1.57), Horotiu (23.1.57), Eltham (24.1.57), Whangaehu (30.1.57), Paekakariki (31.1.57), Hihitahi (18.3.57), and Pahiatua (25.3.57), so would appear to be widespread in the North Island.

Habits

Smith (1931) records it as a pest of lettuce in Europe and as overwintering on currants and gooseberries.

In the present survey it appeared in 2 paddock (10 specimens) and 5 roadside (40 specimens) samples, the greatest number (31) being taken in the roadside sample from Eltham.

Economic Significance

This species is of no obvious importance in pastures, probably being associated with attendant weeds.

He. 8. *Brevicoryne brassicae* (L.)

Distribution

This species is world-wide. Cottier (1953) states "the species is well distributed over the Dominion". In the present survey it was taken at Warkworth (22.1.57), Piopio (24.1.57), Te Matai (4.3.57), Taumarunui (8.3.57) and Greytown (25.3.57).

Habits

This is the well known aphid associated with cruciferous crops.

Economic Significance

The species is of no importance in pastures. Only 7 specimens were taken and these could be accounted for by drift or the presence of cruciferous weeds.

He. 37. *Myzus persicae* (Sulz.)

Cottier (1953) describes this world-wide species as being "ubiquitous and of much economic importance", and records it from 47 host plants in New Zealand. In the present survey it was taken in 2 samples, Norsewood (11.2.57), 1 specimen, and Whakatane (14.2.57), 3 specimens. There is no information available suggesting that it may be of importance in pastures.

He. 38. *Rhopalosiphum splendens* (Theo.)

Distribution

Cottier (1953) records this world-wide species from Auckland (*Hordeum*), Palmerston North (*Lolium*), and Mt. Egmont, 4,000 ft (*Psilopilum*).

Fig. 8 indicates the stations at which this species was taken. It was present in 9 paddock (167 specimens) and 3 roadside (3 specimens) samples, the greatest number being 150 in the paddock sample from Marton on 27.3.57.

Habits

Cottier states "This is a rare species in New Zealand and has not yet been reported to cause damage in the field, although it has proved

injurious to grasses under glass". It appears from records that this species tends to build up late in the season.

Economic Significance

It seems likely that this species does cause considerable damage to pastures. Studies late in the season in ryegrass areas should indicate its relative importance.

He. 39. *Rhopalosiphum padi* (L.)

Distribution

This species is world-wide. Cottier (1953) records it from Aramoho (*Gramineae*), Pukekohe (*Hordeum*), Palmerston North (*Lolium, Triticum, Zea*), Otago (*Avena*), and Taieri (*Phalaris*).

In the present survey it was taken at Eltham (24.1.57), Bulls (30.1.57), Rotorua (4.3.57), Mangapakeha (25.3.57), Marton (27.3.57), and Tarata (2.4.57), the greatest number being an estimated 500 in the sample from Marton. It is thus shown to be widespread in New Zealand.

Habits and Economic Significance

Cottier's remarks are of particular interest. "This species is the common aphid infesting Gramineae in New Zealand. It has been observed severely infesting young wheat about a foot tall. From centres of infestation it spreads in ever widening circles, plants turning yellow and quickly becoming stunted. In the Southland district it has caused similar injury to oats. Although widely distributed the species is, however, seldom a severe pest in the field. It is commonly found on maize, but does not appear to cause noticeable injury. The species is very troublesome on Gramineae grown under glass".

This species is thus suspected of being of importance in pastures, and like *R. splendens* might repay late seasonal studies. In addition to mechanical damage there is the possibility of virus transmission in these common grass-infesting species.

He. 40. *Geoica lucifuga* (Zehn).

This species is world-wide in its distribution. Cottier (1953) records it from the roots of *Danthonia* at Kerikeri. One specimen, an alate, was taken at Opotiki on 14.2.57 in a paddock sample. It is not of economic importance.

He. 41. *Capitophorus elaeagni* (Del Guercio)

This world-wide species has been recorded by Cottier (1953) on *Carduus, Cirsium, Cryptostemma, Cynaea, Elaeagnus* and *Silybum*, from widely scattered localities in the North Island and from the north of the South Island. In the present survey it occurred in paddock samples at Whakatane (14.2.57) and Manaia, Taranaki (3.4.57) in small numbers indicating its probable association with weeds. It is of no importance in pasture.

He. 42. *Aulacorthum solani* (Kltb.)

This species is world-wide. Cottier (1953) records it from 38 host plants in widely scattered districts of both Islands. In the present survey a single specimen was taken in paddock samples at Eltham (24.1.57), and Gisborne (12.2.57). It is not of importance in pastures.

He. 43. *Aphis craccivora* Koch

Cottier (1953) records this world-wide species from 11 host plants at widely separated points in both Islands. It is the common black species infesting legumes. One specimen from a paddock sample at Kerikeri (18.1.57) and 2 from a paddock sample at Warkworth (22.1.57) are recorded. It is not of importance in pastures.

He. 44. *Macrosiphum avenae* (F.)

This is a world-wide species. Cottier (1953) records it from Waitakere and Tararua Ranges (Gramineae), Auckland (*Paspalum* and *Secale*), and Palmerston North (*Rhipogonum*). In the present survey single specimens were taken from paddock samples at Opotiki (14.2.57), Coromandel (6.3.57), and Hunterville (18.3.57). It is not of importance in pastures.

He. 45. *Macrosiphum euphorbiae* (Thos.)

This is a world-wide species. Cottier (1953) records it on 46 host plants from throughout both Islands. In the present survey 5 specimens were taken in a paddock sample from near the Mohaka River (Napier-Taupo road), 15.2.57, and 1 specimen in a roadside sample at Whangaehu on 30.1.57. The species is of no importance in pastures.

He. 46. *Lipaphis erysimi* (Kltb.)

This is a world-wide species. Cottier (1953) records it on crucifers from Waipu to Palmerston North in the North Island. It is a severe pest of stock and occasionally damages other crucifers. In the present survey a single specimen was taken from a roadside sample at Te Matai on 4.3.57.

He. 47. *Macrosiphum sonchi* (L.)

The species is of world-wide distribution. A single specimen was taken in a roadside sample from Paekakariki on 31.1.57. Dr. W. Cottier (pers. comm.) states that this species is found on thistle, and has not previously been recorded from New Zealand.

Hemiptera – Heteroptera

Fam. Cryptostemmatidae

He. 36. *Ceratocombus* sp.

One adult was taken from a paddock sample at Mangapakeha on 25.3.57. Dr. T. E. Woodward (pers. comm.) in identifying the genus states that this species is "almost certainly new to science." The family is not generally regarded as being of economic importance.

Fam. MIRIDAE

He. 10. *Megaloceroea reuteriana* F.B. White.

Distribution

Hutton (1897) records the species from Canterbury. Myers (1926) records it from "North Auckland through Lake Taupo to Wellington", and in the central plateau area at least to 3,000 ft. In the South Island he records it from Governor's Bay and Mt. Grey. Woodward (1954) records the species as being "widespread in Australia and New Zealand".

The distribution encountered in the present survey is shown in Fig. 9. The apparent break between the northern and southern distribution areas may not be so real as would appear here.

Habits

Woodward states that it "is common on grasses, sedges and rushes" and Myers states that it "has been swept from pasture and from marram grass (*Ammophila arenaria* (L.) Link.), the latter being much favoured." Myers also mentions a second species of *Megaloceroea* from cocksfoot at Wanganui in December and January, but was probably confusing a colour variant of *M. reuteriana.*

This species appeared in 23 paddock (125 specimens) and 22 roadside (258 specimens) samples, the maximum catch being 8 adults and 63 nymphs in a roadside sample from a poor pasture area in high

Fig. 9.—Distribution map for the mirid *Megaloceroea reuteriana* He. 10.
Fig. 10.—Distribution map for the mirid *Eurystylus australis.* He. 17.

country near Palmerston North. Nymphs and adults were taken in January, February, and March. No information is available on feeding habits.

Economic Significance

Moderate populations of this insect were encountered, but without further information on feeding habits its importance cannot be determined. It is not suspected of being of importance, however.

He. 17. *Eurystylus australis* Poppius

Distribution

Myers (1926) records this species from Auckland and Nelson. The species also occurs in Australia, and it is thought to have been introduced into New Zealand fairly recently. The distribution encountered in the present survey is shown in Fig. 10.

Habits

Myers records it as a serious pest of the passion-vine (*Passiflora edulis* Sims) and from apple and grasses. In the present survey it was taken in 5 paddock (5 specimens) and 8 roadside (16 specimens) samples, the greatest number being 4 adults in the case of a roadside sample from Opotiki on 14.2.57. Nymphs were taken in January and March, and adults in January, February, and March.

Economic Significance

It is not regarded as being of importance in pastures.

He. 18. *Halticus tibialis* Reuter

Distribution

Woodward (1950) states "This small bug was described from Java (1891) and has since been widely recorded from tropical Africa and Asia (including Ceylon, the Carolines, Amboina and Macassar)". He records for the first time its occurrence in New Zealand from specimens taken in the Bay of Islands.

The distribution encountered in the present survey is shown in Fig. 11. It is seen that it is generally distributed in the northern portions but does not appear to have reached the eastern side of the main ranges. Speed of spread southward from the presumed entry point of Auckland may be slowed down by the species preference for warmer conditions. It would seem to be a comparatively recent arrival.

Habits

It was taken in 27 paddock (196 specimens) and 2 roadside (2 specimens) samples, the maximum number being in a paddock sample from Waitoa on 5.3.57 which contained 20 adults and 12 nymphs. Nymphs were taken in January (North Auckland) and March (majority), and adults in January, February, and March.

Fig. 11.—Distribution map for the mirid *Halticus tibialis*. He. 18.
Fig. 12.—Distribution map for the mirid *Stenotus binotatus*. He. 25.

Economic Significance

The species is recorded as being injurious to beans in the Carolines. It seems unlikely to be of very great importance as a pasture pest although close observation of field habits would be necessary for accurate assessment.

He. 25. *Stenotus binotatus* (F.)

Distribution

This species is widely distributed in Europe and North America. Myers (1926) records it in the North Island from the extreme north to Wellington, and in the South Island from Nelson, Canterbury, and the west coast. The distribution met in the present survey is indicated in Fig. 12, which shows it to be more common in western areas of the North Island.

Habits

Only adults were taken in the present survey. The species occurred in 3 paddock (5 specimens) and 12 roadside (27 specimens) samples, the greatest number being 7 in a roadside sample from Waverley on 24.1.57.

Myers states that "the adult appears in October, is plentiful in December and January, and also occurs in February . . . points to the ovum being the overwintering stage". He indicates that in Europe

and America the insect is essentially a pest, sometimes a serious one, of cocksfoot, ryegrass, and timothy, and states that in New Zealand "it is sufficiently numerous on and destructive to pasture grasses to be considered a pest."

Economic Significance

The foregoing remarks on habits, together with Morrison's (1938) account incriminating the species as an important causal agent of "sticky dough" in wheat, make this species of considerable economic importance. As a pest of pastures, however, it is more likely to be of significance in areas saved for seed production. High populations are probably more common in the South Island.

He. 26. *Deraeocoris maoricus* Woodward

Distribution

Woodward (1950) describing this species, records it from the Bay of Islands, Auckland, and Nelson. In the present survey it appeared in 5 paddock (9 specimens) and 3 roadside (4 specimens) samples taken at Warkworth (22.1.57), Bulls (30.1.57), Tutira (11.2.57), Gisborne (13.2.57), Whakatane (14.2.57), Okere Falls (4.3.57), Waitoa (5.3.57), and Otorohanga (7.3.57). The species is thus widespread in the North Island.

Habits and Economic Significance

Nothing is known of the habits of this species. Only adults were taken. It is not considered to be of economic importance.

He. 27. *Calocoris norvegicus* (Gmelin)

Distribution

This species is widespread, occurring commonly in Europe. It is recorded (Cumber 1953b), following its determination by Dr. T. E. Woodward, in light-trap catches in the Manawatu. It has also been taken at Paihia, Bay of Islands, in early January 1949. The present survey records it from Te Kuiti, Piopio, Waitara, Eltham, and Alton, all on 24.1.57. Nymphs and adults were present, the greatest catch being 5 adults in the Piopio sample.

Habits and Economic Significance

This species appears to be a fairly recent introduction from overseas. All specimens were taken in roadside sweeps. It is not likely to prove troublesome in North Island pastures.

He. 29. *Lopus decolor* (Fallen)

Distribution

Dr. T. E. Woodward (pers. comm.) advises that this is a widespread holarctic species, not previously recorded from New Zealand. It has evidently arrived in the country quite recently, being taken in only one sample—that of the roadside sweep at the top of Bombay Hill, near Pukekohe, on 23.1.57.

Habits and Economic Significance

Only adults (19) were taken. Dr. Woodward advises that "Kullenberg, who has made the most careful biological observations on this species, found it to feed in Sweden mainly on the seed-heads of *Agrostis tenuis* and *A. canina,* and occasionally on other grasses, and also on *Trifolium.* According to some other authors, it lives mainly on composites like *Chrysanthemum* and *Hieracium* (Kullenberg doubts this) and in Germany has been recorded from *Carex* sp. Kullenberg notes it inserting its eggs into the stalks of the panicles of *Agrostis,* and Knight records oviposition in *Juncus* in North America."

It will be interesting to watch the spread of this as yet unimportant pasture species in New Zealand.

He. 30. *Calocoris laticinctus* (F. Walker)

Hutton (1897) records this species from New Zealand. Woodward (1954) records it from the Three Kings Islands taken when sweeping *Blechnum,* grasses, and sedges, and indicates that this species is known only from New Zealand.

The present survey records two adult specimens from a paddock sample at Whangaroa on 17.1.57 in rough *Danthonia* with interspersed bracken country. The writer also has taken this species in southern Manawatu so that it would appear to be widespread in the North Island. It is not important in pastures.

Fam. ANTHOCORIDAE

He. 33. *Xylocoris* sp. possibly *flavipes* (Reuter)

A single specimen was taken in a paddock sample from Napier on 11.2.57. *Xylocoris flavipes* "is quite widespread in the Pacific and elsewhere" (Woodward pers. comm.). This is the first suspicion that the species may be present in New Zealand. However, it may yet prove to be a new species of *Xylocoris.*

Fam. NABIDIDAE

He. 12. *Nabis capsiformis* Germar

Distribution

Woodward (1954) records the species as being "widespread in the Pacific and is now nearly cosmopolitan". Myers (1926) describes it as being "the most abundant and widely distributed nabid in New Zealand". The distribution encountered in the present survey is shown in Fig. 13.

Habits

Nabididae are predaceous and commonly found on herbage where they attack phytophagous insects. Myers records host plants for *N. capsiformis* as weeds, tall grasses, lucerne, red clover, *Juncus,*

Sparganium, and *Cyperus*.

This species appeared in 17 paddock (27 specimens) and 11 roadside (21 specimens) samples, the greatest catch being in a paddock sample at Maramarua on 6.3.57 which yielded 2 adults and 3 nymphs. Both adults and nymphs were taken in January, February, and March.

Economic Significance

This species occurs in relatively small numbers, and the only injury to plants could be through oviposition in stems. It cannot therefore be regarded as a damaging species, and, on the contrary, because of its predaceous nature, must be regarded as a highly desirable and useful species being doubtless instrumental in reducing the numbers of injurious insects.

He. 16. *Nabis maoricus* F. Walker

Distribution

Woodward (1954) indicates that the species is "Endemic to New Zealand, where it is common and widespread". In the present survey the species was taken at Waitara (24.1.57), Kai-Iwi (24.1.57), Himatangi (30.1.57), Hihitahi (18.3.57), Pahiatua (25.3.57), Taueru (25.3.57), Norsewood (26.3.57), and Hunterville (27.3.57).

Habits

There is little specific information, but the same general remarks apply to this as to *N. capsiformis*. Woodward records it from *Lepto-*

Fig. 13.—Distribution map for the nabid *Nabis capsiformis*. He. 12.
Fig. 14.—Distribution map for the lygaeid *Cymus novae-zelandiae*. He. 3.

spermum ericoides and *Myoporum laetum*. This species was taken in 3 paddock (18 specimens) and 5 roadside (13 specimens) samples, the maximum catch being 1 adult and 15 nymphs from the paddock sample at Himatangi. Nymphs and adults were taken in January and March.

Economic Significance

As in the case of *N. capsiformis*, the species is to be regarded as a beneficial one.

Fam. LYGAEIDAE

He. 3. *Cymus novaezelandiae* Woodward

Distribution

This species was described by Woodward (1954), but its presence was noted under the genus *Cymodema* sp. by Myers (1926). Myers and Woodward record it from widely scattered North Island districts, and from Christchurch. This widespread distribution is borne out in Fig. 14 which indicates the survey occurrences.

Habits

According to Myers "Adults have been taken in August, December, and February in the sweeping net, and in May and July in winter quarters at the bases of the rush clumps, where they were most abundant in the later month. Meadow grasses, especially *Bromus unioloides* H.B.K., are favoured. In winter the bases of *Juncus effusus* L. afford them shelter".

In the present survey, the species occurred in 28 paddock (81 specimens) and 4 roadside (7 specimens) samples. The greatest catch was in the case of a roadside sample from Gisborne-Tiniroto on 12.2.57 which yielded 20 adults. Adults and nymphs were taken in January, February, and March.

Economic Significance

It seems probable that the species is of little importance in pastures, although there may be some damage during seed formation. The numbers encountered are low. Habits appear to be similar to those of the species which immediately follows.

He. 9. *Nysius huttoni* F.B. White

Distribution

Hutton (1897) records the species as common in Canterbury and Otago. Myers (1926) records it as widely distributed throughout both Islands and plentiful up to an altitude of 5,000 ft in the Tararua Range. Fig. 15 indicates the distribution met in the present survey.

Habits

Morrison (1938) incriminated this species as one of the causal agents of the condition in wheat which gives rise to "sticky dough".

Gurr (1952) indicated that this species damages crucifer seedlings, and gives a summary of its life history. Adults overwinter and give rise to at least two generations during the following spring and summer. This species thrives where conditions are dry and the vegetation is relatively sparse.

It was taken in 12 paddock (30 specimens) and 12 roadside (64 specimens) samples. The greatest catch was 39 adults and 3 nymphs from a roadside sample taken at Taumarunui on 8.3.57. Nymphs were taken in February and March, adults in January, February, and March.

Economic Significance

It seems probable that any pasture damage is of a minor nature as close turf does not provide the ideal conditions for their congregation and rapid build-up. A study of seed crops would be necessary before their importance in this direction could be ascertained.

Fig.15.—Distribution map for the lygaeid *Nysius huttoni*. He. 9.
Fig. 16.—Distribution map for the pentatomid *Dictyotus caenosus* He. 1.

He. 23. *Pachybrachius nigriceps* (Dallas)

Distribution

Woodward (1954) states that this species "is widespread in the Pacific area". It has been recorded from North Auckland, Greytown, and Wellington. The present survey produced single adults from Helensville (23.1.57), Paekakariki (31.1.57), Wairoa (12.2.57), and Okere Falls (4.3.57).

Habits and Economic Significance

The habits are, apart from its casual association with *Phormium*, *Juncus*, *Carex*, *Scirpus*, *Leptospermum*, and *Lotus*, not known. It is not important as a pest of pastures.

He. 34. *Rhypodes clavicornis* (F.)

Distribution

Myers (1926) indicates that this endemic species is distributed widely from Whangarei to Otago, being present over a considerable range of altitudes. In the present survey, single nymphs were taken at Opotiki and Whakatane on 14.2.57.

Habits and Economic Significance

Myers also indicates that there is probably only one generation each year with overwintering adults. He records it from grasses and a number of forest trees, but there are no grounds for suspecting that it is important in pastures.

Fam. PENTATOMIDAE

He. 1. *Dictyotus caenosus* (Westwood)

Distribution

This species also occurs in Australia, being found in Tasmania as well as on the mainland. Hutton (1897) records it as occurring from Auckland to Otago. Myers (1926) indicates its distribution as being from Kaitaia to Wellington in the North Island, and as being plentiful in Marlborough and Canterbury in the South Island. The distribution met in the present survey is shown in Fig. 16, indicating its presence throughout the North Island.

Habits

D. caenosus overwinters in the adult stage. After mating in spring, eggs are laid on herbage and the nymphs which hatch out develop into adults during the summer months, there apparently being only one brood each year. Myers also states that this species "seems to prefer grasses and low herbage generally. It has been swept from English grasses, including cocksfoot, ryegrass, lucerne, and red clover, and from pastures and roadside grasses . . . found in large numbers sheltering . . . at the bases of rushes".

This species was taken in 15 paddock (28 specimens) and 26 roadside (71 specimens) samples. The greatest catch was in a roadside sample from Whangaehu on 31.1.57 which yielded 7 adults and 8 nymphs. Nymphs and adults were present in January, February and March.

During the examination of dung in pastures, it was found that considerable numbers of adults and nymphs took advantage of the protection afforded by dry pats.

Economic Significance

It seems probable that, in keeping with the habits of phytophagous pentatomids generally, *D. caenosus* draws largely upon developing seed embryos and is most likely to prove damaging in this capacity. Where large populations occur in crops grown for seed, the insect should be regarded with considerable suspicion. The possibility that it causes significant damage in crops of lucerne grown for seed might warrant investigation.

He. 22. *Nezara viridula* (L.)

Distribution

The species is world-wide. It reached New Zealand in 1944 and since then has spread to most parts of the North Island. It has recently appeared in northern portions of the South Island. In the southern portions of its range it is probably restricted to lower areas. In the present survey it was taken at Wellsford (22.1.57), Mokau (24.1.57), Waitara (24.1.57), Whakatane (14.2.57), and Okere Falls (4.3.57).

Habits

These are well known (Cumber 1949, 1951) the insect being a serious pest of vegetables and fruit. Forming seeds in legumes are keenly sought, and undisturbed roadside patches of clover often harbour moderate populations.

Economic Significance

It is not regarded as a potential pasture pest.

He. 24. *Rhopalimorpha obscura* A. White

Distribution

Hutton (1897) and Myers (1926) indicate that this species is present throughout New Zealand. In the present survey it was taken at Te Kuiti (24.1.57), Kai-Iwi (24.1.57), Bulls (30.1.57), and Gisborne-Tiniroto (12.2.57).

Habits

The above records concern nymphs with the exception of the last mentioned. The greatest catch was 5 nymphs at Kai-Iwi. Myers gives considerable data on the life history. He indicates that it overwinters as adults and that there is one generation each year. It occurs in "miscellaneous herbage, long grass, English grasses, sedges. On cocksfoot the nymphs feed with avidity on the fully formed but not yet hardened seeds".

Economic Significance

This species is not regarded as being of importance in pastures.

DISCUSSION

The Hemiptera concerned here include both endemic and introduced species. Some of the endemic species are new to science. The introduced species include some which are unrecorded, and many which have been recorded quite recently.

The pattern of distribution of an introduced species will be related to its port of entry, its period of existence in the country, the climate of the area from which it has come, and the nature of the countryside surrounding the port of entry.

Lopus decolor is probably a very recent introduction with Auckland as the port of entry, for this species was found only at Bombay Hill, Pukekohe.

Halticus tibialis (Fig. 11) may also be a fairly recent introduction. It probably arrived in Auckland and spread northwards in keeping with its preference for warmer habitats. Its southern spread is apparently to a line between Marton and Opotiki. Its further dispersal may also be hampered by the axial mountain chain, so that it may not reach Hawke's Bay for some time.

Rather an interesting distribution is shown by *Megaloceroea reuteriana* (Fig. 9). It would appear that Auckland and Napier have each received this species independently. There is even evidence that the Hawke's Bay population may have spread through to the Manawatu via the Gorge area. However, in view of the relatively limited sampling, a note of caution should be sounded in respect of such hypotheses.

In contrast to the introduced species, those that are endemic, or are known to have been present in the country for a long period, tend to be widespread and without evidence of zoning. However, climatic preferences may be indicated as in the species *Erythroneura zealandica* (Fig. 1), which is more commonly found in the southern portions of the North Island, and, if the trend continues may be of considerable importance in the South Island.

To forecast the importance of a particular insect species is a difficult task, especially in the case of endemic species which in their native habitats are in balance with natural controlling agents. Such a forecast must include a prediction of agricultural trends and managements.

The survey has indicated a number of potential pest species whose real importance may be determined only by closer study. The importance of the species may be governed by any special purpose for which the pasture is required, e.g. early spring feed, or seed crops. Insidious damage may be suspected where there are high populations of phytophagous insects, especially when they are selective feeders and potential virus transmitters.

A study of the jassids will undoubtedly be necessary in New Zealand sooner or later, and not only in respect of pastures. They have received considerable attention in other countries in relation to forage problems. Their pest potentialities have already been indicated, and their ability to defy quarantine measures is well known.

Two aphid species are shown to be capable of considerable build-up in late summer. It would appear that under conditions of normal grazing, pasture is too disturbed to favour such a build-up, but in closed pastures -especially those held for seed there may be considerable damage.

In the mirids and lygaeids there are several potentially important species, but it would appear as in the aphids, that build-up and damage of economic importance may only occur under specialised conditions as in the closing of pastures for seed production.

Acknowledgements

In survey work of the type carried out here, assistance from specialists is unavoidable, and it is also necessary to draw heavily upon existing literature. Dr. W. Cottier, Director, Entomology Division, Department of Scientific and Industrial Research, has identified and provided information on the aphids. Dr. J. W. Evans, Director of the Australian Museum, Sydney, has assisted with the jassids. The greater part of the remaining material was identified by Dr. T. E. Woodward, University of Queensland, Brisbane, whose assistance is especially acknowledged. Field assistance by the staff of the Entomology Division substation at Palmerston North is also gratefully acknowledged.

References

Atkinson, J. D.; Brien, R. M.; Chamberlain, E. E.; Cottier, W.; Dingley, Joan M.; Jacks, H.; Reid, W. D.; Taylor, G. G. 1956: "Plant Protection in New Zealand". 699 pp. Government Printer, Wellington, N.Z.

Cottier, W. 1931: An Insect Survey of Potato Foliage in New Zealand. *N.Z. J. Sci. Tech. 13 (3):* 125-39.

————1953: Aphids of New Zealand. *N.Z. Dep. sci. industr. Res. Bull. 106.* 382 pp.

Cumber, R. A. 1949: The Green Vegetable Bug *Nezara viridula. N.Z. J. Agric. 79:* 563-4.

————1951: The Introduction into New Zealand of *Microphanurus basalis* Woll. (Scelionidae: Hym.) Egg-Parasite of the Green Vegetable Bug, *Nezara viridula* L. (Pentatomidae). *N.Z. J. Sci. Tech. B32 (5):* 32-7.

————1952a: Studies on *Oliarus atkinsoni* Myers (Hem. Cixiidae) Vector of the Yellow-Leaf Disease of *Phormium tenax* Forst. I. Habits and Environment with a Note on Natural Enemies. *Ibid. B34 (2):* 92-8.

————1952b: Studies on *Oliarus atkinsoni* Myers (Hem. Cixiidae) Vector of the Yellow-Leaf Disease of *Phormium tenax* Forst. II. The Nymphal Instars and Seasonal Changes in the Composition of Nymphal Populations. *Ibid. 34 (3):* 160-5.

————1953a: The New Zealand Species of *Oliarus* (Hem. Cixiidae). *Trans. roy. Soc. N.Z. 81 (1):* 71-2.

————1953b: Flight Records of Coleoptera and Hemiptera Taken with a Modified Rothamsted Light Trap Operated at Paiaka. *N.Z. J. Sci. Tech. B34 (4):* 242-4.

————1958: The Insect Complex of Sown Pastures in the North Island. I. The General Picture Revealed by Summer Sweep-Sampling. *N.Z. J. agric. Res. 1:* 719-49.

Gurr, L. 1952: Notes on *Nysius huttoni* F.B. White, a Pest of Wheat in New Zealand. *N.Z. Sci. Rev. 10 (7):* 108-9.

Hutton, F. W. 1897: Synopsis of the Hemiptera of New Zealand which have been Described Previous to 1896. *Trans. N.Z. Inst. 30:* 167-87.

Morrison, G. 1938: Surveys of the Insect Pests of Wheat Crops in Canterbury and North Otago during the Summers of 1936-37 and 1937-38. *N.Z. J. Sci. Tech. A20 (3):* 142-55.

Myers, J. G. 1922: The Order Hemiptera in New Zealand. With Special Reference to its Biological and Economic Aspects. *Ibid. 5 (1):* 1-12.

————1923: A Contribution to the Study of New Zealand Leaf-hoppers and Plant-hoppers (Cicadellidae and Fulgoroidea). *Trans. N.Z. Inst. 54:* 407-29.

————1924: The New Zealand Plant-hoppers of the Family Cixiidae (Homoptera). *Ibid. 55:* 315-26.

————1926: Biological Notes on New Zealand Heteroptera. *Ibid. 56:* 449-511.

Osborn, H. 1939: "Meadow and Pasture Insects". 288 pp. The Educators' Press, Columbus, Ohio.

Paterson, C. R. 1947: A Recent Outbreak of Honey Poisoning. IV. The Source of Toxic Honey. Field Observations. *N.Z. J. Sci. Tech. A29: (3):* 125-9.

Quinton, R. J. 1956: The Forage Insect Problem. *Conn. agric. Exp. Sta. Circ. 197.* 14 pp.

Smith, K. M. 1931: "A Textbook of Agricultural Entomology". 285 pp. C.U.P.

Tillyard, R. J. 1926: "The Insects of Australia and New Zealand". 560 pp. Angus and Robertson, Sydney.

Tuthill, L. D. 1952: On the Psyllidae of New Zealand (Homoptera). *Pacific Science 6 (2):* 83-125.

Wolcott, G. N. 1937: An Animal Census of Two Pastures and a Meadow in Northern New York. *Ecol. Monogr. 7:* 1-90.

Woodward, T. E. 1950: New Records of Miridae (Heteroptera) from New Zealand, with Descriptions of a New Genus and Four New Species. *Rec. Auck. Inst. Mus. 4 (1):* 9-30.

————1954: New Records and Descriptions of Hemiptera-Heteroptera from the Three Kings Islands. *Ibid. 4 (4):* 215-33.

EFFECTS OF INGESTION OF PARAFFINS BY RUMINANTS

V. BLOOD PLASMA PROTHROMBIN TIMES OF COWS DRENCHED WITH HEAVY LIQUID PARAFFIN

By C. S. W. Reid* and H. E. Hutchings†

Summary

A dose of 75 ml of heavy liquid paraffin (British Pharmacopoeia) given twice daily for 26 days to 3 cows in the declining stages of lactation, had no detectable effect on the plasma prothrombin times as measured by a modification of Quick's one-stage method using human brain as the source of thromboplastin.

The same dose rate of oil given to 7 cows in late pregnancy, or late pregnancy and the first two weeks after calving, was also without effect.

The average prothrombin time in the first experiment was 23.0 sec (48 samples) and in the second experiment, 21.6 sec (145 samples). These figures were of the order of values found by other investigators for bovine plasma, but were rather higher than those for the normal human-plasma standards (14–16 sec).

Introduction

Liquid paraffins are effective anti-foaming agents for the treatment and prevention of bloat in cattle (Reid and Johns 1957). However, in non-ruminants, the ingestion of mineral oils may result in undesirable side effects, the most prominent of which is interference with the absorption of essential fat-soluble substances in the diet, including fatty acids, carotene, and vitamins A, D, E, and K. Apparently the non-digestible paraffin entrains a proportion of these materials, which becomes lost to the animal when the oil is excreted in the faeces. The effect is most marked in the case of carotene; and, while the greatest interference is caused by liquid paraffin, other paraffins and poorly digested "fatty" substances have a similar action (McGillivray and Worker 1958). In other papers in this series (McDowall *et al.* 1957; McGillivray 1957; McGillivray *et al.* 1959) it is shown that, in ruminants also, ingestion of paraffin oil depresses the absorption of carotene and vitamin E. The possible effect on the vitamin K status of cows is dealt with in the present paper.

Vitamin K is essential for the maintenance of normal blood clotting. Deficiency of the vitamin, whether due to an inadequate intake or poor absorption from the gut, results in an increasing prolongation of the clotting time. A tendency develops to bleed easily, and fatal haemorrhage may ensue from minor injury.

An observation that rats on a diet containing 20% mineral oil developed a haemorrhagic tendency led Elliot *et al.* (1940) to experi-

* Plant Chemistry Division, Department of Scientific and Industrial Research, Palmerston North.
† Pathology Department, Palmerston North Public Hospital.

N.Z. J. agric. Res. 2: 26–34.

ment with the effect of this diet on blood clotting. It was found that continued ingestion was associated with the development of a progressively longer blood clotting time. This effect was completely reversible by the parenteral administration of vitamin K. An improvement in clotting, but not a return to normality, occurred if vitamin D were given, but vitamin A was without effect. Similar results were obtained by Barnes (1942) using mice, and in this case normal blood clotting was restored by intraperitoneal injections of menadione, a synthetic compound with strong vitamin K activity. However, in a trial with rabbits, no significant changes in blood clotting occurred after a period of four weeks on a diet containing 20% mineral oil (Barnes *loc. cit.*).

That pregnancy might predispose the subject to the effects of ingestion of liquid paraffin is suggested by the report of Javert and Macri (1941) of an increase in blood clotting time of some pregnant women following dosing with 15–30 ml of the oil daily or intermittently. Again the effect could be corrected by vitamin K given parenterally, but if the vitamin was given orally, it was relatively ineffective unless the administration of the oil was stopped simultaneously.

Compared with the proportions used by Elliot and her co-workers and by Barnes, the quantities of oil used in New Zealand for the treatment and prevention of bloat—up to 180 ml/cow/day, usually 90–120 ml—represent only a very small proportion of the diet. However, they do approximate to the proportions in Javert and Macri's cases. Further, as is reported in other parts of this series, the ingestion of 150 ml of liquid paraffin results in a profound reduction of carotene absorption and to a lesser extent, of vitamin E absorption (McDowall *et al* 1957; McGillivray *et al.* 1959). It was considered advisable, therefore, to determine whether or not ingestion of mineral oil at this rate was followed by an increase in blood clotting time, particularly in cows in the last stages of pregnancy.

The measurement of the natural whole-blood clotting time is not satisfactory as a practical routine test. Also, since clotting is a complex process* with a number of substances involved in consecutive reactions, the interpretation of results is difficult. In vitamin K deficiency, the lengthened clotting time is apparently due to the reduction of the concentrations of two substances in the blood, prothrombin and the so-called Factor VII. Prothrombin has a major role in clotting. In the penultimate stage, in the presence of thromboplastin, calcium, Factor VII, and other substances, it is quantitatively converted to thrombin, the activator for the final stage, transformation of fibrinogen into fibrin.

In 1935, Quick devised a simple test, the "one-stage prothrombin time" which consists of adding a potent "thromboplastin" preparation to the plasma and then measuring the clotting time of the mixture

* The nomenclature and interpretations used in this paper are those of Biggs and MacFarlane, 1957, who critically review present knowledge of blood clotting, particularly of human blood.

when an excess of calcium is added. This was first assumed to be a measure of the concentration of prothrombin in the blood but it is now known that the test in fact measures the activity of a number of substances involved in clotting, including both prothrombin and Factor VII. While this limits the interpretation of positive results, it is generally agreed that where prothrombin times are normal, abnormality of any of the factors contributing to the test is unlikely. Despite non-specificity and other defects (Link 1948; Biggs and MacFarlane 1957), the Quick test and its modifications have proved of considerable value in work on coagulation problems, including vitamin K deficiency. It was considered adequate to reveal any gross effects of ingestion of mineral oil in the present investigation.

This paper gives the results of the measurements of prothrombin times of blood samples taken during the paraffin drenching trials reported by McDowall *et al.* 1957 and by McGillivray *et al.* 1959.

Methods

Experiment 1

One member of each of three sets of lactating identical-twin dairy cows was drenched twice daily with 75 ml of heavy liquid paraffin* (British Pharmacopoeia grade) for 26 days. Each treated animal thus received a total of 3.9 l of oil. Treatment was carried out after morning and evening milking.

The experiment was divided into three periods:

1. Pre-treatment period: 21-25 March, 1956.
2. Treatment period: 25 March-20 April.
3. Post-treatment period: 20-22 April.

Blood samples for the measurement of prothrombin times were taken at weekly intervals, extra samples being taken during the pre-treatment and the last week of the treatment periods.

The animals were grazing a ryegrass/white-clover/red-clover pasture.

Further details of the experiment are given by McDowall *et al.* 1957.

Experiment 2

Ten unrelated, pregnant cows were divided into three groups and received the following treatments:

A. Untreated control group.

B. Drenched with heavy liquid paraffin for 14–21 days before calving and 14 days after calving.

C. Drenched with paraffin before calving only.

* The oil used in both experiments was Stan-Vac White Oil 890, kindly supplied by the Vacuum Oil Co. (N.Z.) Ltd.

The dose rate was again 75 ml twice daily, with the exception of one animal in group C (cow 109) which received 150 ml twice daily.

Details of cows, treatment and calving dates are given in Table 1.

Blood samples for measurement of prothrombin times were taken 3 times weekly for two weeks before and after calving, but usually less frequently at other times.

During the experiment, the cows were grazing a grass-dominant ryegrass/white clover pasture.

Further details of this experiment are given by McGillivray *et al.* (1958).

TABLE 1. DETAILS OF TREATMENTS IN EXPERIMENT 2

Group	Cows	Dose Rate	Treatment Starting Date	Number of Days Treated	Total Quantity of Oil Given (litres)	Calving Date
A Controls	37	—	—	—	—	22/7/57
	40	—	—	—	—	13/8
	57	—	—	—	—	28/7
B Oil before and after calving	41	75 ml twice daily	19/7/57	29	4.35	2/8
	60		5/7	38	5.7	28/7
	185		5/7	31	4.65	22/7
C Oil only before calving	59	75 ml twice daily	22/7	22	3.3	21/8
	85		5/7	30	4.5	16/8
	87		19/7	13	1.95	2/8
	109	150 ml twice daily	10/8	10	3.0	22/8

Blood Sampling Technique

Blood was obtained from the external jugular vein by the normal open-needle method, using 14-gauge needles about 3 cm long.* The sample for prothrombin-time measurement was taken only if the blood had flowed freely for 5–10 sec in a continuous jet from the needle.

Sampling procedure was as follows: The plunger was removed from a dry 2-ml glass syringe† and the open barrel filled from the blood jet, a 19-gauge needle restricting outflow through the nozzle. To reduce frothing, the blood was made to run down the inner side of the syringe during this process. When the barrel was full, the plunger was carefully replaced, excess blood ex-

* "Diapoint" needles, manufactured by Chas. F. Thackray Ltd., Leeds, England, were found particularly satisfactory.

† Type BD 2YL Yale Luer-Lok manufactured by Becton Dickinson and Co., Rutherford, N.J., U.S.A.

pelled, and the last 1.8 ml run gently into a small sample tube containing 0.2 ml of 3.8% tri-sodium citrate solution. After stopping with a rubber bung, the sample tube was gently turned end over end for 30 seconds.

Plasma was obtained by centrifugation.

Immediately after use, all apparatus in contact with blood was rinsed with saline and then stored under water until cleaning. Pure soap flakes were used for washing and generous rinsing with tap and distilled water preceded drying in a hot oven. Detergents were avoided as they readily cause haemolysis of red cells and alteration to prothrombin times. The syringe barrels were greased with a trace of light paraffin (B.P.).

Measurement of Prothrombin Times

The plasma prothrombin times were measured by the modified Quick one-stage method described by Biggs and MacFarlane (1957). Dried acetone-extracted human brain (Biggs and MacFarlane *loc. cit.*) was used as the "Thromboplastin", the concentration being 0.5 g of the powder in 10 ml of normal saline. With this preparation, the clotting time of known-normal human plasma was 15 ± 1 sec. The technique had been in routine use for several years in the Haematology Department of the Palmerston North Public Hospital.

Results and Discussion

The results are shown in Table 2 (Exp. 1) and Fig. 1 (Exp. 2).

TABLE 2. Prothrombin Times, Paraffin Drenching Experiment 1

Period	Sampling Date	Prothrombin Times (sec)							
		Individual Cows						Averages	
		25	26*	93	94*	97	98*	Control	Treated
Pre-experimental	21/3	29	27	24	25	25	27	26	26
	23/3	23	23	22	28	23	23	23	25
Experimental	28/3	23	29	23	23	25	25	24	26
	4/4	24	20	20	20	20	21	21	20
	11/4	21	25	22	20	22	22	22	22
	18/4	27	25	27	22	24	23	26	23
	20/4	24	24	22	21	21	21	22	22
Post-experimental	27/4	19	22	20	20	20	19	20	20

Treated animals* drenched 75 ml White Oil 890 at each milking from the evening 25.3.56 to the morning 20.4.56.

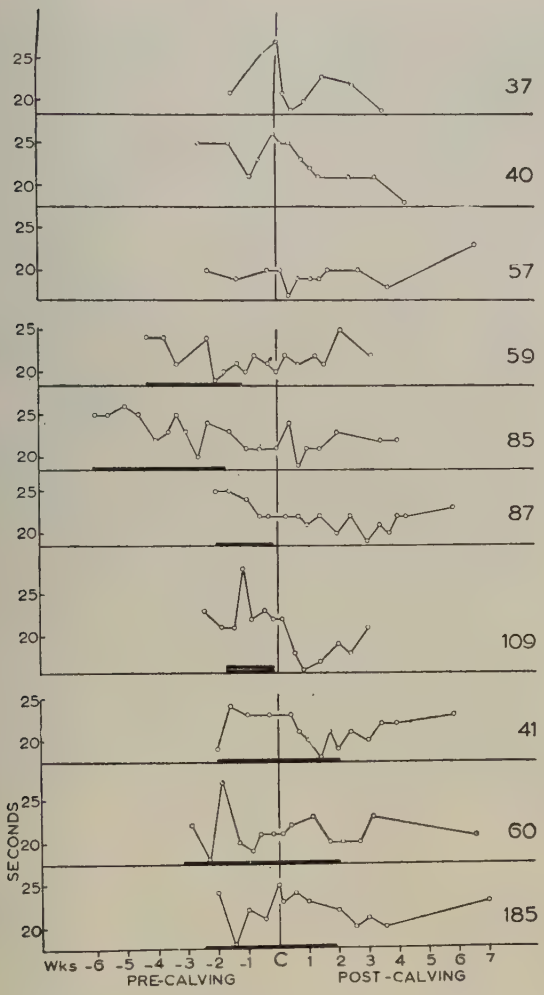


Fig. 1.—Plasma prothrombin times of cows in Experiment 2, plotted in relation to calving (C). Heavy line indicates period of treatment with liquid paraffin.

Duplicate sub-samples were measured from every blood sample; the results were rarely more than 2 sec apart.

The time elapsing between sampling and testing was of the order of 1–2 h. This delay did not appear to effect the results.

In neither experiment did the administration of liquid paraffin appear to have any deleterious effect on the plasma prothrombin times. In Exp. 2, the prothrombin times of 4 cows (Nos. 41, 59, 85, 87), actually *decreased* during treatment. Samples taken from 3 of the

calves* about 12 h after birth had prothrombin times with ± 2 seconds of those taken at the same time from their mothers (cows 40, 41, 109). Had the mothers been affected, it seems likely that the clotting times of the calves would have been markedly prolonged (cf. "sweet-clover disease"—Schofield 1924).

A feature of both experiments was the degree of variation in the prothrombin times of individual animals as compared with the relative stability of the prothrombin times of known-normal human subjects. The cause was not established, although considerable trouble was taken to try and eliminate all likely factors. Some of the changes appeared to be systematic trends taking place over a period of days or even weeks, while others appeared to be random. In the first experiment, there was a general decrease in clotting times of all animals during the course of the trial. In the second experiment, clotting times tended to be longer for samples taken during July while a distinct decrease in prothrombin time followed calving in a number of animals.

The prothrombin time of cow's plasma was consistently longer than that of normal human plasma, the average clotting time in Exp. 1 being 23.0 and in Exp. 2 21.6 sec as against 14–16 sec for the standards. These times were of the order of those found for cattle by

TABLE 3. COMPARISON OF PUBLISHED PROTHROMBIN TIMES (sec) FOR LARGE DOMESTIC ANIMALS

Investigators	Thromboplastin	Cattle	Sheep	Goat	Horse	Pig
Dougherty *et al.* (1949)	Bovine lung		13–25			
Kment (1950)	Rabbit brain	22.4	17.3		26.6	16.1
Lenz-Doroschkin (1955)	Rabbit brain	27.8	14.8	16.8	17.7	15.8
Buruiana and Rauchbach (1956)	Pig lung	26.2*	15.4	15.4	23.8	19.7
		19.2†				
Reid and Hutchings	Human brain		23.02 ± .38 (Exp. 1) 21.64 ± 0.24 (Exp. 2) (Mean) (S.E.)			

* Buffalo
† Ox

other workers (Table 3). However, inter-specific comparisons using a single technique must be made with caution, for if the conditions are not optimal, particularly if the thromboplastin is unsuitable, abnormal clotting times will be obtained. Thus Quick (1937) found that the prothrombin time of chicken plasma was 45–60 sec when a rabbit-brain thromboplastin was used, but only 11 sec when a chicken-brain or -lung thromboplastin was used. Buruiana and Rauchbach (1956) found

* No other calves were tested as all were over 24 h old at the first opportunity for bleeding.

figures of 44.5 sec for fowl plasma, and 31.6 sec for human plasma (i.e. twice the figure obtained in the present experiments for normal human subjects). This suggests that their pig-lung thromboplastin was unsuitable for the latter species. Clearly results obtained by the use of a standard technique should be checked by tests using a thromboplastin prepared from the individual species being investigated.

The apparent lack of effect of the treatment on the plasma prothrombin times in these experiments contrasts with the marked changes in the absorption of carotene and vitamin E that were occurring simultaneously (McDowall *et al.* 1957; McGillivray *et al.* 1959). However, a normal prothrombin time merely indicates that the animal is not deficient in vitamin K. No information is given as to whether absorption of the vitamin is being affected. In the present experiment, if absorption *was* affected, either the animals were able to draw on body reserves, or the depression of absorption was insufficient to interfere with the acquiring of sufficient of the vitamin to maintain normal clotting. As ruminants have a luxury supply of vitamin K, this last explanation may well be the correct one. Not only is there a high intake, green leaves being one of the richest sources of the vitamin, but also large amounts are synthesised by the rumen micro-organisms (McElroy and Goss 1940). It seems probable, therefore, that ruminants can tolerate high intakes of heavy liquid paraffins before any adverse change in blood clotting occurs.

Acknowledgements

The authors are indebted to Dr. T. H. Pullar, Pathologist, Palmerston North Public Hospital, for his interest and helpful criticism. Our thanks are also due to Mr. P. Vlieg of Plant Chemistry Division for technical assistance and to the farm staff of the Dairy Research Institute (N.Z.) who carried out the drenching.

References

Barnes, W. A. 1942: *Proc. Soc. exp. Biol., N.Y. 49:* 15.

Biggs, R.; MacFarlane, R. G. 1957: "Human Blood Coagulation and its Disorders". 2nd ed. Blackwell Scientific Publications, Oxford.

Buruiana, L. M.; Rauchbach, K. 1956: *Mh. Vet. Med. 2:* 83. (*Vet. Bull. 26:* 3598).

Dougherty, R. W.; Conner, G. H.; Hoyo Migaki 1949: *Amer. J. vet. Res. 10:* 327.

Elliot, M. C.; Isaacs, B.; Ivy, A. C. 1940: *Proc. Soc. exp. Biol., N.Y. 43:* 240.

Javert, C. T.; Macri, C. 1941: *Amer. J. Obstet. Gynec. 42:* 409.

Kment, A. 1950: *Wien. tierärztl. Mschr. 37:* 461. (*Vet. Bull. 32:* 3042).

Lenz-Doroschkin, I. 1955: Inaug. Diss. Munich. (*Vet. Bull. 26:* 3285).

Link, K. P. 1948: Blood Clotting and Allied Problems. *Trans. 1st Conf. Josiah Macy, Jr. Foundation, N.Y.*

McDowall, F. H.; McGillivray, W. A.; Reid, C. S. W. 1957: *N.Z. J. Sci. Tech. A38:* 839.

McElroy, L. W.; Goss, H. 1940: *J. Nutr. 20:* 527.

McGillivray, W. A. 1957: *N.Z. J. Sci. Tech. A38:* 878.

McGillivray, W. A.; McDowall, F. H.; Reid, C. S. W. 1959: *N.Z. J. agric. Res. 2:* 35.

McGillivray, W. A.; Worker, N. A. 1958: *N.Z. J. agric. Res. 1:* 267.

Quick, A. J. 1935: *J. biol. Chem. 109:* lxxii.

————1937: *Amer. J. Physiol. 118:* 260.

Reid, C. S. W.; Johns, A. T. 1957: *N.Z. J. Sci. Tech. A38:* 908.

Schofield, F. W. 1924: *J. Amer. vet. med. Ass. 64:* 553.

EFFECTS OF INGESTION OF PARAFFINS BY RUMINANTS

VI. INGESTION OF HEAVY LIQUID PARAFFIN BY COWS NEAR PARTURITION IN RELATION TO MILK YIELD AND COMPOSITION AND TO FAT-SOLUBLE VITAMIN CONTENT OF THE BUTTERFAT DURING THE EARLY STAGES OF LACTATION.

By W. A. McGillivray,[1] F. H. McDowall,[2] and C. S. W. Reid[3]

(*Received for publication, 21 August 1958*)

Summary

Cows were treated prior and subsequent to parturition with 150 ml of heavy liquid paraffin B.P. daily, a dose rate previously found effective for the control of bloat. The treatment had no effect on milk and fat yields. It did not cause any change in the levels of carotene, xanthophyll, vitamin A, and tocopherol in the butterfat in the first milk drawn after parturition but it caused in subsequent samples a marked increase in the rate of post-parturition fall in levels of carotene, vitamin A ester and tocopherol, and a slight increase in the rate of fall of xanthophyll level. It did not affect the vitamin A alcohol content of the butterfat.

Because of its marked effect on the colour of early post-colostral butterfat, treatment of cows with heavy liquid paraffin for bloat control could be expected to lower the depth of colour of spring-made butter.

The treatment with paraffin caused marked decreases in the levels of carotene and tocopherol in the blood, which, near parturition, were superimposed on the changes in the levels normally occurring at that time.

There was no evidence of any effect of the paraffin treatment on the health of the cows before, during, or after parturition. The only result of any nutritional importance was the reduced vitamin potency of the colostrum for calf feeding.

Introduction

In a previous trial (McDowall *et al.* 1957) it was found that the drenching of cows with 75 ml of heavy liquid paraffin (B.P.) twice daily caused a marked reduction in the absorption of carotene and tocopherol from the feed consumed. These effects were evident as decreases in the levels of carotene, vitamin A ester, and tocopherol in the blood, and as parallel reductions in the carotene, vitamin A, and tocopherol contents of the butterfat. Xanthophyll absorption was not affected. There were no detectable effects on the yield of milk, on the fat and solids-not-fat contents of the milk, or on the iodine and saponification values of the butterfat.

[1] Massey Agricultural College and The Dairy Research Institute (N.Z.), Palmerston North.

[2] The Dairy Research Institute (N.Z.), Palmerston North.

[3] Plant Chemistry Division, Department of Scientific and Industrial Research, Palmerston North.

The dose rate then used was of the order of that likely to be given for the treatment and prevention of bloat (Reid 1955; Reid and Johns 1957). Subsequent work (McGillivray 1957a) showed that the effects on absorption of fat-soluble substances were proportional to the dose rate of mineral oil when this rate was varied over the range of 30 to 300 ml per day. The results of these two trials indicated that, with a diet high in carotene and fat-soluble vitamins, little harm would be caused to the health of lactating cows by dosing with mineral oil for as long as four weeks at the rate normally used in the treatment and control of bloat. The first trial was in autumn when the cows were in the later stages of lactation. The second trial was in late spring when the cows were in the second and third months of lactation. Thus in both cases lactation was well established before commencement of treatment.

The periods immediately before and after parturition are however more critical for the health and nutrition of the animal, and at these times factors such as reduced absorption of fat-soluble nutrient, resulting from dosing with mineral oil, could possibly produce more drastic effects than were observed previously. Near parturition there are heavy drains on the body reserves both to supply the demands of the late-term foetus and to provide the large quantities of calcium, protein, carotene, vitamin A and other substances present in the colostrum and early lactation milk. This results in marked decreases in the content of these substances in the blood, despite high dietary intakes, as, for example, of carotenoids from pasture (Thompson and McGillivray 1957). This situation may be aggravated by normal farming practice where, as a result of the best grass being conserved for the already lactating cows, the diet of cows before calving frequently contains a large proportion of hay and is therefore relatively low in essential fat-soluble nutrients.

Under New Zealand farming conditions most cows calve before the main period of bloat incidence, but there may be a number of late-calving cows in each herd and these would graze pastures sprayed with mineral oil for bloat prophylaxis. The present trial was undertaken therefore to determine whether treatment with mineral oil before and after calving had any apparent adverse effect on the health of the cows and on the establishment of normal lactation.

The experiment was also designed to investigate the fat-soluble-vitamin content of the colostrum and the early milk particularly from the point of view of the nutrition of the young calf, which is almost entirely dependent on colostrum for its initial body reserves of these materials.

The effect of paraffin treatment on the carotene content of early milk is of interest also to the buttermaker. Variations in the carotenoid content of New Zealand butter are mainly seasonal in nature and are not associated with stage of lactation (McDowell and McDowall 1953) but the high carotenoid content, and hence rather intense yellow colour, of butter manufactured in the early spring has been attributed in part to the presence in the butter of a relatively high proportion of early lactation fat (McDowell 1956).

The present paper describes the effects of mineral oil dosing at the rate of 150 ml per day for various periods before and after calving on the carotenoid, vitamin A, and tocopherol contents of the blood and milk. Possible effects on vitamin K absorption are discussed in an earlier paper (Reid and Hutchings 1959), and use is made of the same data in a paper dealing with the origin of milk carotenoids and vitamin A (McGillivray, in press).

Experimental

Suitable pairs of monozygotic twins were not available from The Dairy Research Institute herd. It was necessary, therefore, to use unrelated cows. They were grazed on grass-dominant ryegrass/white-clover pastures throughout the experimental period, viz, July-September 1957. Nine cows, selected for uniformity and for closeness of expected calving dates, were divided into three groups, as follows:

Group A. Untreated controls.

Group B. Drenched with heavy liquid paraffin for 14–21 days before calving and for 14 days after calving.

Group C. Drenched with heavy liquid paraffin before calving only.

With Group C it was planned that the treatment with heavy liquid paraffin should be over the period 21 days before calving to 2–3 days before calving, but owing to errors in predicting the calving dates the actual dosing period for the cows commenced 30, 43 and 14 days, and ended 9, 11 and 2 days, before calving. The three cows thus were dosed for 21, 32 and 12 days, respectively.

For the treated cows the daily dose rate was 150 ml of heavy liquid paraffin* given as a drench of 75 ml twice daily after milking. This dose rate was known to be effective for bloat control, and was that used in the first investigation (McDowall *et al.* 1957).

After parturition, the milk yields from each cow were measured daily.

Night and morning composite milk samples were collected daily for the first three days after calving, and thereafter at 2- or 3-day intervals for approximately 12 days. A further 1-day sample was taken at approximately one month after calving. All these samples were analysed for fat and solids-not-fat contents.

From the milk samples collected during the first three days of lactation, butterfat was extracted by the method of Olson *et al.* (1939). Thereafter until the end of the experimental period it was isolated by churning of cream obtained by centrifugal separation from milk collected on the same day as the samples for assay were taken (Dolby 1954).

Blood samples were taken from all cows at regular intervals (usually 2–3 days) throughout the experimental period. Butterfat samples were

* The oil used was Stan-Vac white oil "890", kindly supplied by The Standard-Vacuum Oil Company N.Z. Ltd.

assayed for carotenoids, vitamin A alcohol and ester, and tocopherol, and blood plasma samples for carotenoids, vitamin A alcohol, and tocopherol. Carotenoids and vitamin A were estimated using the method of Thompson *et al.* (1949), and tocopherol as described by Worker (1957).

RESULTS

As stated above, the cows were not monozygotic twins and were not paired. Some variation in the results within each group could therefore be expected. Since, however, the curves for the results for all cows within each group showed similar trends, there appeared to be sufficient basis for presentation of the results as averages for each group. For the results for cows of Group C there were some difficulties in application of this method of presentation, owing to the wide difference in the periods of treatment of the individual cows with heavy paraffin in relation to time of parturition (see above).

Yields of Milk, Butterfat, and Solids-not-fat

The average daily yields of milk and butterfat for the cows in each group over the period of the investigation are shown in Fig. 1. The yields of both milk and fat for the cows in Group B were slightly

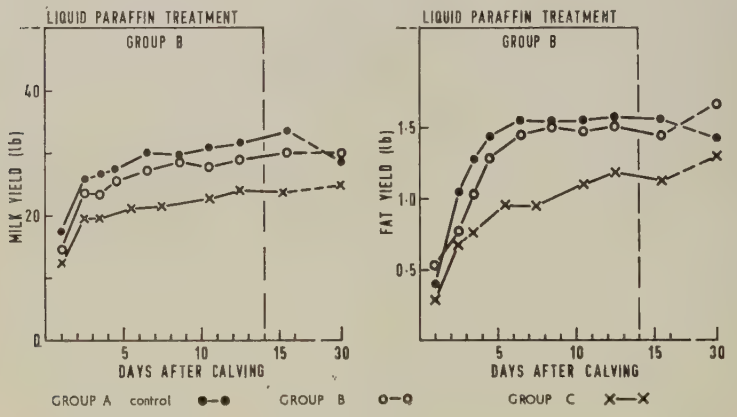


Fig. 1.—Daily yields of milk and fat over the first thirty days of lactation.

lower than those for the control cows, but since the cows in the two groups were unrelated, differences of this order of magnitude cannot be regarded as significant. The curves for the two groups showed similar trends (Fig. 1). This may be taken as a further indication that the treatment with heavy liquid paraffin before and after calving did not affect the production of milk and butterfat. Similarly there was no significant effect of treatment on the solids-not-fat production.

The average yields of milk, fat, and solids-not-fat for Group C were much lower than for Groups A and B. This difference was due

to the low yields from two of the cows in Group C. The production curve for the third cow in Group C was closely similar to those for Groups A and B. Since the production for the first two cows of Group C remained consistently low throughout the season, and since the yield curves shown in Fig. 1 for Group C were of the same general shape as those for Groups A and B, it is concluded that the low average yield for Group C was not in any way related to the treatment of the cows in this group with heavy liquid paraffin.

Carotenoid, Vitamin A, and Tocopherol Contents of Butterfat

From the results in Table I it is apparent, particularly for Groups A and B, that with the exception of xanthophyll, the contents of these

TABLE 1. Average Levels of Various Constituents in the Milk Fat at Intervals though the Experiment (μg/g fat)

Days after Calving	½			12½			30+		
Constituent	Group A	B	C	Group A	B	C	Group A	B	C
Carotene	81	97	71	13	8.0	15	17	14	16
Xanthophyll	12	32	27	5.4	7.3	7.6	5.0	7.2	6.7
Vitamin A ester	22	25	27	7.9	4.7	8.1	6.9	7.0	7.6
Vitamin A alcohol	2.0	2.1	2.5	0.30	0.33	0.52	0.13	0.20	0.32
Total vitamin A potency (I.U./g)	215	252	216	49	30	54	51	47	53
Tocopherol	180	195	230	46	24	51	45	51	48

substances in the fat were fairly uniform in the first samples after parturition and in the samples taken 30 days later (when for Group B treatment had already been discontinued for 16 days). The lack of uniformity in xanthophyll levels is in agreement with previous findings that cows differ widely in the xanthophyll content of their butterfat (McGillivray, unpublished results). The effect of heavy liquid paraffin in depressing the levels of carotene, vitamin A ester, and tocopherol in the milk fat is apparent from the figures shown for Group B 12½ days after calving.

In Fig. 2, the results have been plotted as percentages of the contents in the initial (½-day) colostral fat sample. This method of presentation admittedly is open to criticism, because the content of these substances in the butterfats from different cows shows its greatest variability at the very start of lactation. In the present instance, however, it appears to be justified by the similarity in initial milk and fat yields and in the initial concentration of the various constituents in the butterfat, and by the fact that the trends in yields and in fat-soluble vitamin contents of the butterfats from the cows within each group did not vary greatly from the group average.

The curves in Fig. 2 show that the values for carotene, vitamin A ester, and tocopherol, computed as set out above, decreased more rapidly

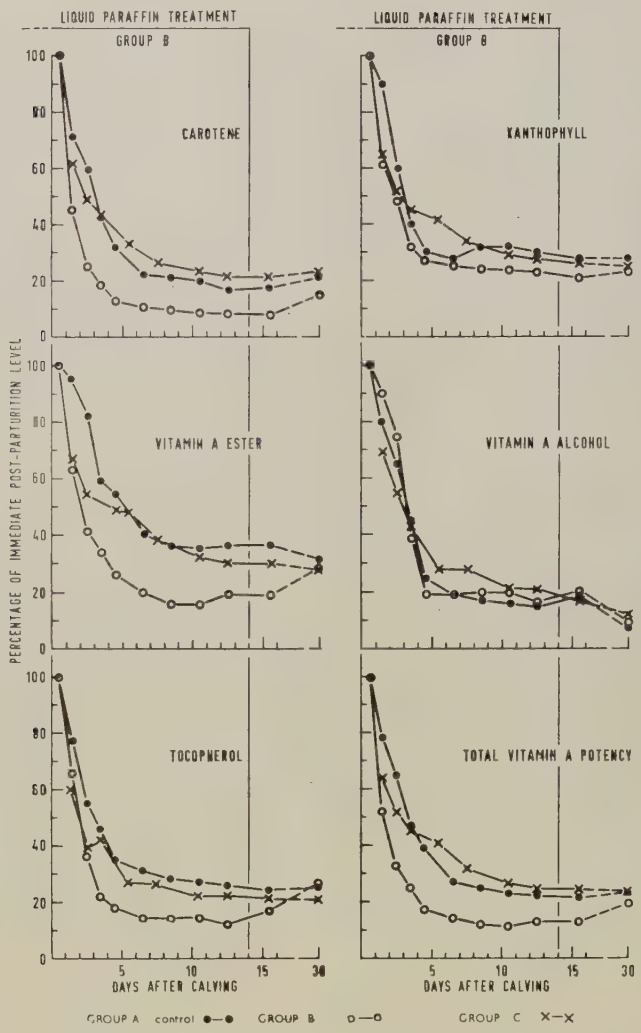


Fig. 2.—Carotenoid, vitamin A and tocopherol contents of butterfat during the first thirty days of lactation. (Expressed as percentage of results for the first sample of butterfat obtained after calving.)

in the butterfat from the cows in Group B than in the butterfat from the control cows, Group A. The curve for xanthophyll also showed a slightly more rapid fall for the group treated with liquid paraffin, but the difference was not large and could have been due to differences in initial levels of xanthophyll in the fat. The levels and changes in the levels of vitamin A alcohol in the fat were not affected by the treatment.

The curves for average contents of carotenoids, vitamin A ester, and tocopherol in the fat for the cows in Group C showed trends intermediate between those for Group A and Group B. In those cows of Group C for which paraffin dosing had been terminated 9–11 days before calving, the curves followed those for the control group except

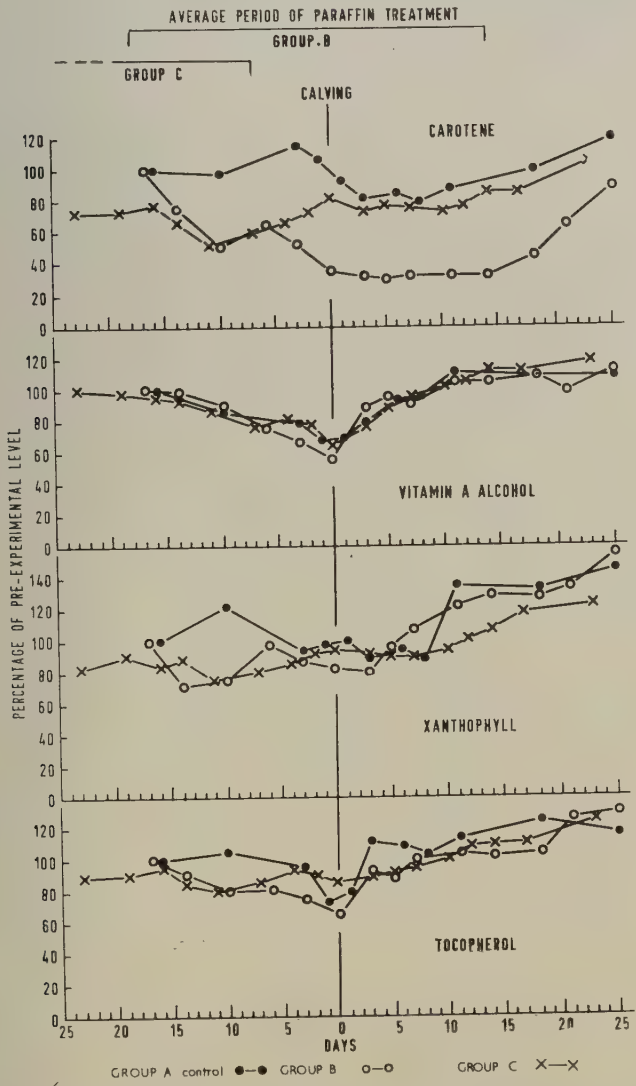


Fig. 3.—Carotenoid, vitamin A alcohol and tocopherol contents of the blood from cows near parturition. (Expressed as percentage of results for pre-experimental blood samples.)

for a slightly more rapid fall during the first few days of lactation. For the cow dosed until 2 days before calving, the curves were closely similar to those for the curves of Group B in the first 3–5 days of lactation, but thereafter they flattened sharply and approached those of Group A. Vitamin A alcohol contents decreased at rates not significantly different from those shown in Groups A and B.

Carotenoid, Vitamin A Alcohol, and Tocopherol Contents of the Blood Plasma

These are shown in Fig 3. To facilitate comparisons, results have been expressed as percentages of pre-experimental levels. The pre-experimental levels represented normal levels for the blood constituents investigated and, as has been found previously, varied considerably from animal to animal. All cows within each group did however show similar trends both on drenching with heavy liquid paraffin and during the period near parturition. Average pre-experimental levels of carotenoids, vitamin A alcohol, and tocopherol in the blood for the three groups are shown in Table 2.

TABLE 2. Average Pre-experimental Levels of Various Constituents in the Blood of Cows in Separate Groups

	Content in the Blood Plasma (μg/100 ml)		
	Group A	Group B	Group C
Carotene	1550	1800	1800
Xanthophyll	140	120	200
Vitamin A alcohol	27	22	31
Tocopherol	600	550	750

The cows in Group A showed, near parturition, characteristic decreases in the contents of carotenoids, vitamin A alcohol, and tocopherol in the blood. A sharp rise in levels of carotenoids in the blood plasma preceded the falls at parturition. Vitamin A alcohol levels commenced to fall earlier than carotenoid or tocopherol levels, and did not exhibit any preliminary rise. These changes, except for tocopherol which was not determined previously, are all similar to those reported by Thompson and McGillivray (1957) for comparable pasture-fed cows.

In Groups B and C, paraffin treatments resulted in decreases in the contents of carotenoids and tocopherol in the blood similar to those already reported for cows in more advanced stages of lactation (McDowall *et al.* 1957; McGillivray 1957a). The average depressions in blood values due to paraffin treatment were 50% for carotene, 25% for xanthophyll, and 20% for tocopherol. The depression in xanthophyll content was apparent only during the paraffin-dosing periods prior to calving. Paraffin treatment after calving, as in the previous investigations, had no apparent effect on xanthophyll absorption. Vitamin A alcohol levels were not affected by paraffin treatment.

Near parturition, these two groups showed, superimposed on the paraffin effects, trends similar to those of the control cows in Group A. Carotenoid contents, depressed by the paraffin treatment, exhibited, shortly before parturition, characteristic increases followed by further decreases to minimum levels at or immediately following parturition. In Group B, carotenoid levels remained low until paraffin treatment ceased 14 days after parturition. The cows in Group C, where paraffin treatment had ceased some days before parturition, calved with increasing contents of carotenoids and tocopherol in the blood. This largely masked any pre-parturition carotenoid increases, but the cows all showed typical depression in blood values at calving. Vitamin A alcohol contents in the blood showed almost identical trends for the three groups.

Discussion

As has been pointed out above, the period near parturition is a somewhat critical one in the nutrition and metabolism of the cow, and it might be anticipated that any adverse effects of treatments such as the dosing with heavy liquid paraffin would be more apparent at this time than at later stages of lactation. In the present investigation, the dosing of cows with heavy liquid paraffin immediately before and after parturition did not affect either the milk or fat yields or the rate at which production increased during the first month of lactation. The indications are therefore that treatment with oil close to time of calving does not affect the health of the cows. This is supported by the absence of any observable untoward effects on the health of the cows and their calves, and is in agreement with previous findings (McDowall *et al.* 1957; McGillivray 1957a) for cows in more advanced stages of lactation.

The depressing effect of the ingestion of heavy liquid paraffin on the carotene content of butterfat as found in the present investigation for cows at the onset of lactation was greater than that found previously for cows at later stages of lactation. A possible explanation for this may lie in differences in the nature of blood precursors of the fat-soluble vitamins in the mammary secretions produced at the onset of lactation as compared with the origins of these vitamins later in lactation. Thus McGillivray (1957b) has shown that after normal lactation is established the carotene and vitamin A in the milk are mainly of immediate dietary origin and are derived through the uptake of blood chylomicrons by the mammary gland. At the onset of lactation, however, some of the milk carotene and vitamin A originates from the uptake by the gland of protein-bound forms of these substances from the blood. Similar conisderations may apply to the secretion of tocopherol.

The fact that the initial contents in the colostrum of all the constituents except xanthophyll were uniform for all the cows would suggest that the colostrum obtained at the first milking had probably been building up for some time and that its fat-soluble vitamin content was virtually independent of immediate dietary intakes and blood contents. That the three groups of cows showed closely similar declines in the contents of vitamin A alcohol in the colostrum and early milk

fat is confirmation of the suggestion that the viamin A alcohol in the blood is the only source of vitamin A alcohol in the milk (McGillivray 1957b). Similarly the more rapid fall in colostral and milk-fat vitamin A ester levels in Group B than in the control group would suggest that the contribution of blood vitamin A alcohol to milk vitamin A ester levels is a very small one even at the onset of lactation. This is not unexpected in view of the relatively low levels of vitamin A alcohol in the blood (25 to 35 μg/100 ml plasma).

The effect of the ingestion of liquid paraffin on the carotene content of the milk and hence on the colour of butter is of considerable interest to the butter manufacturer. Clearly, the effect on the colour of early milk fat is greater than that found at later stages of lacation. For its effect on the colour of commercial butter, it is the quantity of carotene secreted over the early stages of lactation which is important. In Fig. 4, the effects of liquid paraffin treatment on the daily secretion

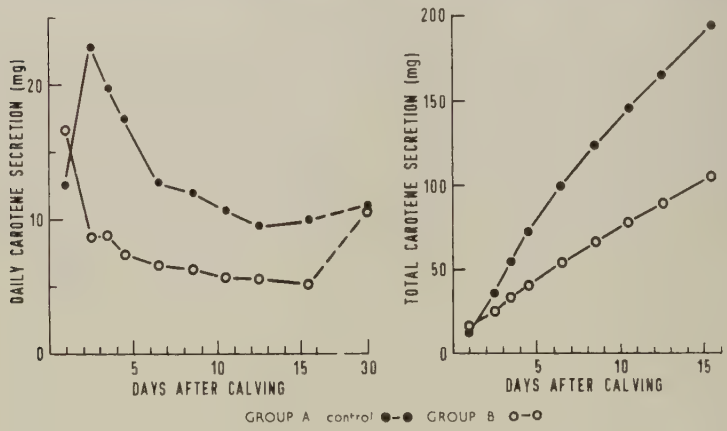


Fig. 4.—Daily and total secretions of carotene over the early stages of lactation.

of carotene and on the total quantity of carotene secreted over the first 15 days of lactation are shown. The paraffin treatment reduced the total quantity of carotene secreted during these 15 days to approximately 50%. Hence the dosing of cows with heavy liquid paraffin before and after calving could have a very marked effect on the colour of the butter produced during the spring months. The extent of this effect would depend on the spread of calving times in commercial herds.

Attention must be drawn to nutritional aspects of the subject, particularly the use of colostrum from paraffin-treated cows for the rearing of calves. Calves are born with almost no reserves of fat-soluble vitamins and are entirely dependent on the high concentrations normally present in colostrum to establish reasonable body reserves. While the effects of the ingestion of heavy liquid paraffin on the fat-soluble vitamin content of the milk may be regarded as nutritionally

relatively unimportant at later stages of lactation, the very marked reductions in the secretion of carotene, vitamin A, and tocopherol could seriously affect the nutritive value of colostrum for the young calf. This could well prove the limiting factor in the use of liquid paraffin for the control of bloat in cows near to time of parturition.

That the dosing of cows with heavy liquid paraffin before and after calving accentuates the decreases which normally occur at parturition in the contents of carotenoids and tocopherol in the blood has already been discussed. In Group B this resulted in the carotene content of the blood being reduced to 30% of normal at parturition. This low level was maintained until treatment ceased 14 days later. It could be presumed that the blood vitamin A ester content would be similarly reduced, indicating a relatively serious reduction in the amount of vitamin A being formed in the intestinal walls. In this connection it is of interest to note that the cows with low contents of carotene and tocopherol in their blood prior to paraffin treatment showed in general the greatest depressions in blood levels on dosing with paraffin near parturition. This would seem to indicate the importance of the nutritional status of the animal prior to paraffin treatment. However, in the absence of precise information regarding the significance even of the normal changes which occur near parturition in the blood levels of fat-soluble vitamins and in which cows show very considerable individual variability, it is not possible to assess the importance of the added effects of paraffin treatment. In view of the very high carotene and tocopherol intakes of pasture-fed cows throughout the year and the rapidity with which the contents of carotenoids, vitamin A, and tocopherol in the blood return to normal after paraffin treatment ceases, it is not considered that the results of the present experiment provide any strong evidence for regarding the period near parturition as more critical for paraffin treatment than any other time, in relation to the fat-soluble vitamin metabolism of the cow itself.

Conclusion

The present series of experiments (Reid 1957; McDowall *et al.* 1957; McGillivray 1957a; Reid and Hutchings 1959; and this paper) has clearly established that the main effect of the ingestion by cows of liquid paraffin of medicinal grade at dose rates likely to be used in the treatment or prevention of bloat is a depression in the absorption of fat-soluble vitamins. The main issue in connection with the use of mineral oil, therefore, is to determine whether this decreased absorption of fat-soluble vitamins during the period of an outbreak of bloat in a herd* could lead to vitamin deficiencies severe enough to affect the health of the cows or to reduce seriously the nutritive value of their milk.

From these investigations the following general conclusions have been reached:

* Usually not more than six weeks but in some areas, e.g. newly developed country in Rotorua and Taupo, the pastures may be bloat-producing for several months.

(1) Because of the high intake of fat-soluble vitamins from pasture throughout the year, it is unlikely that cows would suffer any ill-effects from the reduced intakes during periods over which heavy liquid paraffin might be administered for the control or prevention of bloat.

(2) Paraffin treatment causes a marked decrease in the nutritive value of colostrum, and hence colostrum from cows dosed with liquid paraffin should not be used as the sole diet of young calves.

(3) Although any reduction of the nutritive value of milk for human consumption is admittedly undesirable, the effects of dosing with liquid paraffin are relatively small and the economic advantage of this oil, for treatment and prevention of bloat, outweigh the nutritional disadvantage resulting from its use.

(4) Heavy liquid paraffin may safely be used for control and prevention of bloat at dosage rates of up to 150 ml per day for periods of at least six weeks.

Acknowledgements

The authors are indebted to Mr. L. P. Hobbs and the farm staff of The Dairy Research Institute (N.Z.), who were responsible for the drenching and the collection of samples; to members of the staff of the Chemistry Laboratories of The Dairy Research Institute and the Biochemistry Department of Massey Agricultural College: and to Mr. P. Vlieg of Plant Chemistry Laboratory for technical assistance.

References

Dolby, R. M. 1954: *J. Dairy Res. 21:* 78.
McDowall, F. H.; McGillivray, W. A.; Reid, C. S. W. 1957: *N.Z. J. Sci. Tech. A38:* 839.
McDowell, A. K. R. 1956: *J. Dairy Res. 23:* 111.
McDowell, A. K. R.; McDowall, F. H. 1953: *J. Dairy Res. 20:* 76.
McGillivray, W. A. 1957a: *N.Z. J. Sci. Tech. A38:* 878.
———1957b: *J. Dairy Res. 24:* 352.
Olson, F. R.; Hegsted, D. M.; Peterson, W. H. 1939: *J. Dairy Sci. 22:* 63.
Reid, C. S. W. 1955: *Proc. 17th Conf. N.Z. Grassl. Ass.*
———1957: *N.Z. J. Sci. Tech. A38:* 825.
Reid, C. S. W.; Hutchings, H. E. 1959: *N.Z. J. agric. Res. 2:* 26.
Reid, C. S. W.; Johns, A. T. 1957: *N.Z. J. Sci. Tech. A38:* 908.
Thompson, S. Y.; Ganguly, J.; Kon, S. K. 1949: *Brit J. Nutr. 3:* 50.
Thompson, S. Y.; McGillivray, W. A. 1957: *J. Dairy Res. 24:* 108.
Worker, N. A. 1957: *J. Sci. Fd. Agric. 8:* 442.

BLOAT IN CATTLE

XI. THE FOAMING PROPERTIES OF PROTEINS, SAPONINS, AND RUMEN LIQUOR

By J. L. Mangan, Plant Chemistry Division, Department of Scientific and Industrial Research, Palmerston North.

(*Received for publication, 17 July 1958*)

Summary

The foaming properties of rumen liquor, bovine salivary mucoprotein, cytoplasmic protein from red clover, and saponins from various pasture legumes have been examined.

Foam strengths of the saponin foams have pH optima, those from red clover and lucerne being between pH 4.5 and 5.0. The presence of calcium in the saponin is necessary for the formation of rigid foams.

Foams from red-clover cytoplasmic protein show maximum strengths at pH values ranging from 5.4 to 6.0. The foaming properties are markedly dependent on salt concentration.

Salivary mucoprotein has greatest foam strength above pH 7.5, but some samples have a secondary peak at pH 6.5.

Rumen liquor from cows fed on red clover has a pH optimum for foam strength at pH 5.4 to 5.7 similar to cytoplasmic protein. The particulate matter in rumen liquor has considerable anti-foaming properties.

The evidence indicates that cytoplasmic protein is of major importance as a foaming agent in causing bloat in cattle. The implications of these results are discussed with reference to bloat.

Introduction

It has been established by previous papers in this series (Johns 1954: Reid and Johns 1957) that the primary cause of bloat in cattle grazing legume pastures in New Zealand is foaming of the rumen ingesta. Fermentation gases produced in the rumen, which are normally eliminated by eructation, are partially retained in the form of a stable foam. Intra-ruminal pressure rises rapidly because of the inability of the belching mechanism to cope with the foam, and unless the pressure is relieved it may culminate in the death of the animal. Johns (1954) suggested that the foaming system in the rumen could be composed of saliva, saponin, and protein, surface-active materials which would enter the rumen of an animal fed on clover. Lindahl *et al.* (1954) have shown that large doses of lucerne saponin will produce bloat in sheep, particularly if the animals had been grazing clover pastures before treatment. Ferguson and Terry (1955) considered that the bloat-producing factor in lucerne juice is a saponin but failed to produce bloat in sheep with pure lucerne saponin.

A further step in the investigation of bloat is the positive identification of the causative foaming agent and a study of the physicochemical requirements necessary for stable foam formation. Clark

(1947) in his comprehensive work on firefighting foams has studied the foaming properties of complex protein hydrolysates and commercial saponins. Little, however, has been published on the foaming properties of well-defined substances. Cumper (1953), using a dynamic foam meter similar to that of Foulk and Miller (1931), showed that pepsin and insulin have maximum foaming power at a pH slightly more alkaline than their isoelectric points, but that foams from β-globulin did not markedly respond to pH changes. The foaming capacity increased with increasing ionic strength. Perri and Hazel (1947) showed that the foaming capacity of soya-bean protein dispersions had an optimum at about pH 4, the approximate isoelectric point, and that both the nature and concentration of cations affected the foaming properties.

There is no reference in the literature to measurements of the foaming properties of the triterpenoid saponins or cytoplasmic proteins of legumes, or of salivary mucoprotein. The present paper reports an investigation into the foaming properties of these compounds and makes comparison with those of the rumen liquor of bloating cows.

Methods

Measurement of Foaming Properties

The methods and nomenclature used are those previously described (Mangan 1958), and except where stated otherwise the foams were generated with air.

Dynamic foam stability is estimated by passing gas at a standard pressure and rate of flow through a sintered glass sparger in a glass tube containing the solution to be examined. The rate of foam expansion is plotted and compared with a perfect foaming solution, such as 0.5% yucca saponin, which gives a straight-line expansion. At a standard time of 15 min, foam height is calculated as a percentage of the height obtained with the perfect foaming solution. Dynamic foam stability is thus expressed as "% perfect stability".

Foam expansion is defined as the maximum number of volumes of foam obtained from one volume of solution under the conditions described above.

Foam strength is measured by the rate of fall of a perforated brass weight (120 g) through the foam generated under the conditions employed above. Foam strength is inversely proportional to the rate of fall and is expressed as 100/slope of the plotted line, i.e. 100 $\times$ time of fall/distance dropped.

Preparation of Saponin Extracts

Attempts to isolate purified saponin from red clover were not successful. The cholesterol method of Walter *et al.* (1954) yielded no saponin when applied to red clover. Other methods of purification such as absorption on carbon, partition between butanol and water, and precipitation at 0°c with 35% acetone at pH 4.5, yielded products

whose foaming properties were partially destroyed. Extracts containing the saponin in an undegraded form and free from other known foaming substances were simply prepared by the following method using ether cytolysis (Chibnall 1939):—Red-clover tops were harvested and immediately sprinkled with a liberal quantity of ether in a stainless steel container. The material was mixed thoroughly and in a few minutes the whole mass was cytolysed by the ether vapour. The mass was placed in a hydraulic press and on application of moderate pressure the vacuolar fluid readily flowed from the tissue. This fluid, comprising about 70% by volume of the moisture content of the herbage, was filtered and stored at 2°c, at which temperature it was stable for several weeks in the presence of the ether. The extracts were found to be free from proteins and peptides, but contained small-molecular-weight compounds such as sugars, amino-acids and mineral salts. Before use, ether was removed by stirring gently in an open dish with a rapid stream of air drawn across the surface. Although the extracts were not purified further for foaming experiments, small-molecular compounds may be removed by dialysis, and the saponin remains in the dialysis sac.

Preparation of Cytoplasmic Protein of Red Clover

The method of Lyttleton (1956) was used with slight modification to obtain the whole cytoplasmic protein rather than a pure component. Red clover was cut and immediately ground in an end-runner mill at +2°c with phosphate buffer pH 7.5, I = 0.1. Cell debris was removed by squeezing through muslin followed by centrifuging at 10,000 g for 30 min. Anhydrous sodium sulphate (11% W/V) was added at room temperature to cause incipient precipitation of protein and aggregation of the remaining chloroplast fragments. After high speed centrifuging at 10,000 g, the whole cytoplasmic protein was precipitated by the addition of sodium sulphate at 25% W/V. The centrifuged protein was then dissolved in phosphate buffer pH 7.5, dialysed against water at 2°c until free from salt and stored at −20°c. Nitrogen was estimated by the micro-Kjeldahl method of Chibnall *et al.* (1943).

Preparation of Bovine Salivary Mucoprotein

Saliva was collected from individual cows by means of a perforated metal tube fitted in the mouth round the lower jaw of the animal under the tongue and connected to a receiver and vacuum pump (Reid unpublished). On application of gentle suction, considerable amounts of saliva were readily obtained. Collections were made while the animals were eating with no apparent contamination of the samples from plant material even when feeding on lush red clover. The samples thus obtained were very viscous.

The saliva was filtered through glass wool, adjusted to pH 5.5 with acetic acid, and the mucoprotein precipitated by the addition of 32% W/V anhydrous sodium sulphate at 30°c. The precipitated protein was filtered off, dissolved in a small volume of sodium bicarbonate solution and the viscous solution obtained dialysed against water at 2°c until free from salt.

From chemical analyses carried out in this laboratory (in preparation), it appears that saliva thus collected is predominantly the submaxillary and residual secretions, and contains a relatively small proportion of parotid saliva. The latter, which is secreted in large volume, has been found to contain only traces of protein and contributes little to the foaming capacity of the mixed saliva. The surface active mucoprotein is found in the submaxillary and residual secretions. The latter secretion is considered to be derived mainly from the sublingual glands.

Rumen Liquor

Permanently fistulated cows were used under a feeding regime described previously (Johns 1954). Samples were withdrawn from the rumen, squeezed through muslin and rapidly cooled with ice to arrest fermentation. This product is termed crude rumen liquor. Clear rumen liquor, free from plant fragments and micro-organisms was obtained by centrifuging for 30 min at 10,000 g in a Spinco preparative ultracentrifuge using a pre-cooled rotor.

Results

Saponins

Effect of pH. Saponin extracts of red-clover leaves were prepared as described above. The pH of a series of aliquots of the same solution was adjusted by the addition of HCl or NaOH and diluted to a convenient volume. Foam strength, as shown in Fig. 1, responded markedly to changes in pH. Above pH 6 foam strength was nil, but rises below pH 5.5 to a sharp peak with an optimum in the region of 4.5 to 5.0. On further acidification foam strength dropped rapidly to almost nil at pH 4.0. Foam expansion shows a similar optimum in the same pH region but not to the same marked extent as foam strength. Thus above pH 6 red-clover saponin gives an appreciable volume of foam which, however, exhibits little resistance to mechanical stress.

Lucerne saponin extracts (Fig. 1) showed an effect of pH on foam strength almost identical with that of red clover. Crystalline lucerne saponin has been shown (Mangan 1958) to give a similar pH/foam strength curve, except that the pH optimum is shifted slightly towards the acid side. This close agreement in foaming behaviour indicates that the extracts prepared by ether cytolysis contain only saponin as the active foaming agent. At pH 4, where foam strength decreases rapidly, both red clover and lucerne extracts precipitate saponin, which redissolves on raising the pH. White-clover and subterranean-clover saponin extracts (Fig. 1) also show responses to pH changes, with maximum foam strength at pH 4.5 and 5.5 respectively.

Effect of Calcium Precipitants. In attempts to isolate a pure saponin from red-clover extracts prepared by ether cytolysis, it became apparent that calcium was a component of the saponin complex which markedly affected the properties of the foams produced. In Table 1 the effect of calcium precipitants is shown on a lucerne saponin extract. In all cases foam expansion was virtually unaffected, but foam strength

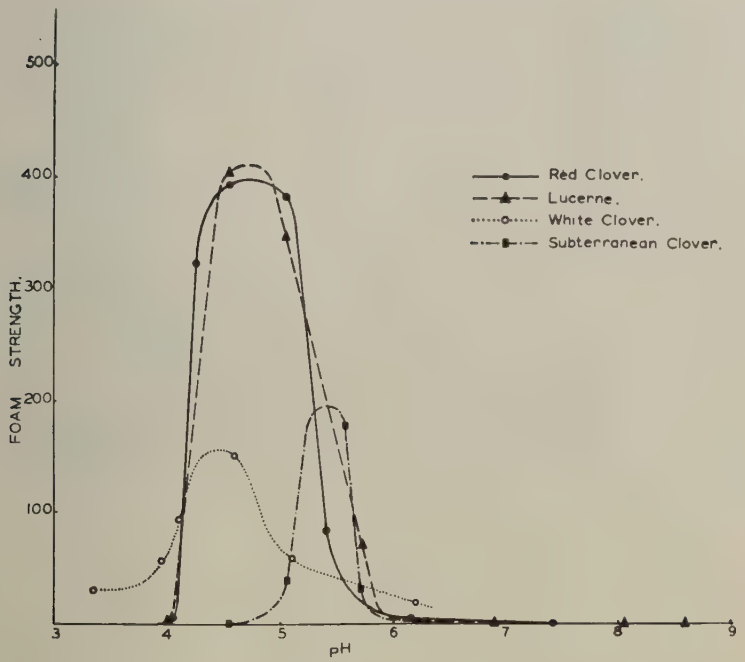


Fig. 1.—The effect of pH on the foam strength of saponins from pasture legumes.

TABLE 1. EFFECT OF CALCIUM PRECIPITANTS ON LUCERNE SAPONIN EXTRACT

Treatment	Foam Strength (100 × Time/Distance)
1. Made alkaline, buffered to pH 5.0 with acetate	923
2. Made alkaline, buffered to pH 5.0 with citrate-phosphate	384
3. Made acid, buffered to pH 5.0 with acetate	362
4. Made acid, buffered to pH 5.0 with citrate-phosphate	2.9
5. Oxalic acid, buffered to pH 5.0 with acetate	0.8
6. Sodium oxalate, buffered to pH 5.0 with acetate	0.3
7. Ethylene diamine tetra-acetate + acetate buffer pH 5	11.6

was in some cases reduced from a very high figure to practically zero.

The lucerne saponin molecule contains two carboxyl groups (Walter, *et al.* 1954) and it could be postulated that the calcium salt occurs as a chain structure with divalent calcium linking the saponin molecules through the carboxyl groups, whereas the sodium salt could only exist as the single molecule. Although there seems no doubt that

calcium is necessary to give a strong saponin foam, the addition of calcium salts or calcium hydroxide to the acid-precipitated saponin did not give a recovery of foam strength, indicating that calcium may not be present in the saponin with simple salt linkages.

Cytoplasmic Protein from Red Clover

Effect of Salt Concentration. A series of protein solutions containing 20 mg N/100 ml, which had been dialysed free from salt, was foamed after the addition of salt ranging from 0 to 0.5 M NaCl. With no salt present, only 4 cm of foam was obtained with no foam strength. As little as 0.063 M NaCl increased foam expansion to the full capacity (45 cm) of the apparatus and dynamic foam stability to 100%. The effect of further increases in salt concentration could not be observed with these measurements. Foam strength, however, increased steadily with increase in salt concentration. Fig. 2a gives the rates of fall of the weights dropping through the foams and Fig. 2b shows that between 0 and 0.33 M NaCl there is an approximately linear increase in foam strength. This, however, increases disproportionately at 0.5 M NaCl possibly owing to a salting out of protein at the interface.

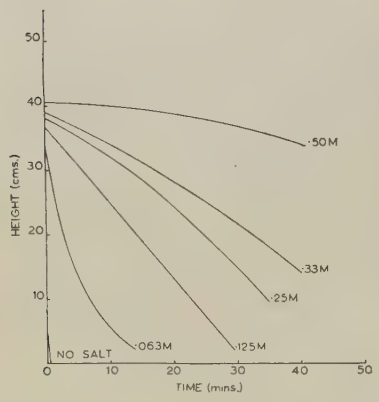


Fig. 2a.

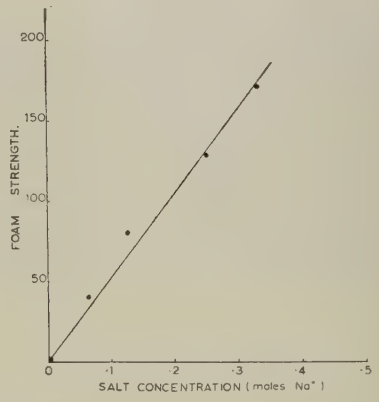


Fig. 2b.

Fig. 2.—Foam strength of red-clover cytoplasmic protein as affected by salt concentration. (a) The plots of the weights dropping through the foams. (b) Foam strength as a function of salt concentration.

Effect of pH. The protein was diluted to 10 mg N/100 ml, made 0.1 M with sodium acetate and the pH adjusted by the addition of acetic acid. Foam expansion and dynamic foam stability were very high over the whole pH range and showed no maxima. Foam strength as shown in Fig. 3 had a marked optimum at pH 5.4. Above pH 6 and below pH 5, although the solution foamed satisfactorily, the foam strength was negligible by the present method.

At higher levels of protein and salt concentration the pH optimum and the rising part of the foam strength curve shifts to a higher pH

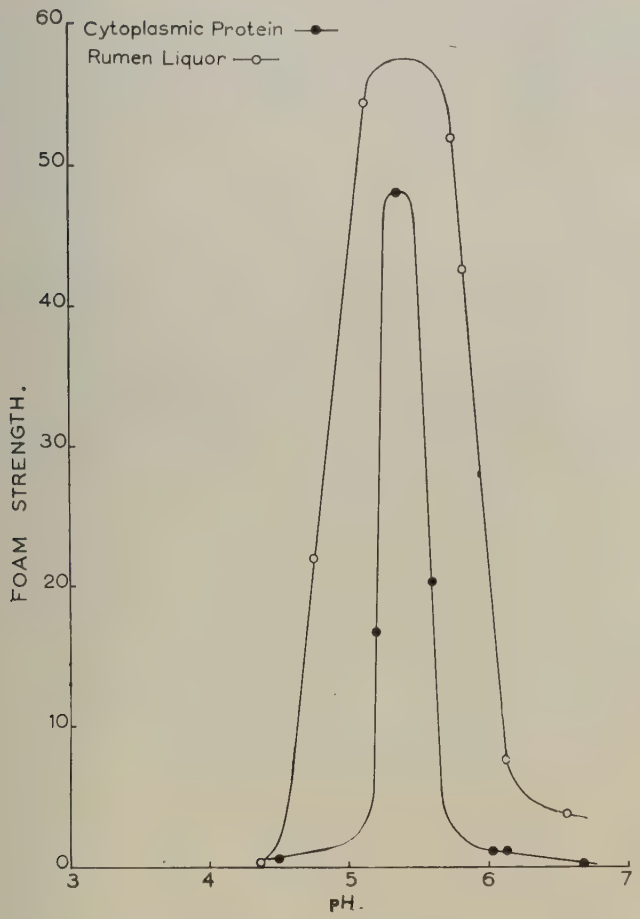


Fig. 3.—The effect of pH on the foam strength of red-clover cytoplasmic protein (10 mg N/100 ml in 0.1M sodium acetate buffer) and clear rumen liquor from a cow bloated after feeding on fresh red clover.

value. As shown in Fig. 4 at a protein level of 20 mg N/100 ml and a salt concentration of 0.5 M, the foam strength curve commences to rise rapidly at pH 6.5 and the pH optimum has shifted to 6.0.

Effect of Cation Species. The cations present in highest concentration in the rumen liquor are sodium and potassium, with smaller amounts of calcium and magnesium. Ammonium ion is also present in appreciable concentrations. Perri and Hazel (1947) have shown that for soya-bean protein dispersions divalent cations are more effective than monovalent species in promoting foaming properties. In Table 2, comparison is made between cytoplasmic protein in sodium

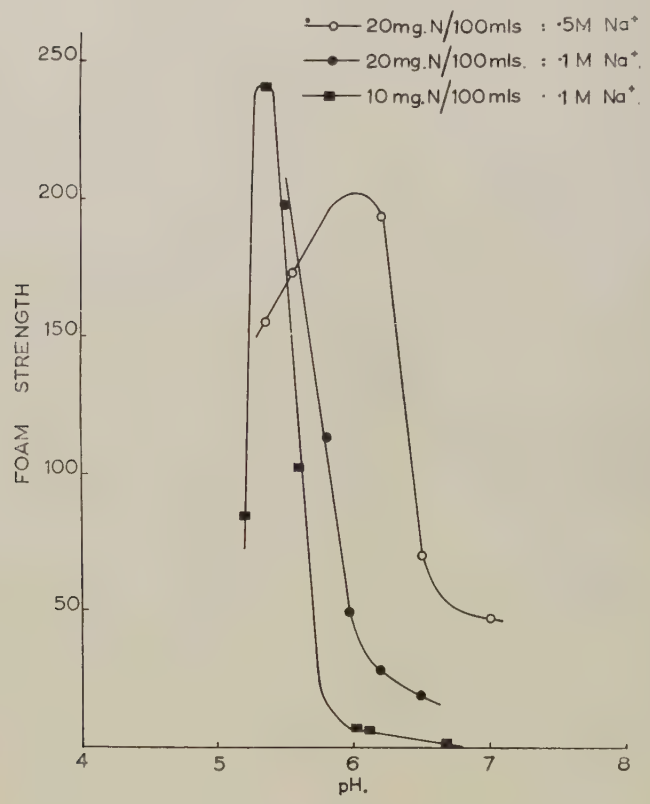


Fig. 4.—Foam strength/pH curves of red-clover cytoplasmic protein at different salt concentrations and protein levels.

TABLE 2. COMPARISON OF THE EFFECTS OF SODIUM AND CALCIUM IONS ON THE FOAMING PROPERTIES OF RED-CLOVER CYTOPLASMIC PROTEIN
(25 mg N/100 ml)

Cation Concentration (moles)	pH	Foam Strength (100 × Time/Distance)	
		Sodium	Calcium
0.0001	6.0	Nil	Nil
0.001	5.5	32.2	61.4
0.01	5.6	32.6	60.0‡
0.10	5.9	149	524*

‡ Slight opalescence.

* Definite precipitation of protein.

acetate and calcium acetate buffers at pH 5.5. Expressed as moles of cation, calcium is about twice as efficient as sodium in promoting foaming properties but on an equivalent weight basis foam strength figures are approximately the same and no special significance can be attributed to the divalent cations, until their concentration reaches the relatively high value of 0.1 M.

Salivary Mucoprotein

Effect of pH. As salivary mucoprotein is a very efficient foaming agent, the standard solution used in these experiments contained only 5 mg N/100 ml. The solutions were made 0.1 M with sodium acetate and the pH adjusted with acetic acid. Dynamic foam stability was very high over the whole pH range shown, and foam expansion similarly was the maximum measurable in the apparatus. Foam strength had a maximum at alkaline pH values with an optimum above pH 8 as shown in Fig. 5. Other samples of saliva which were

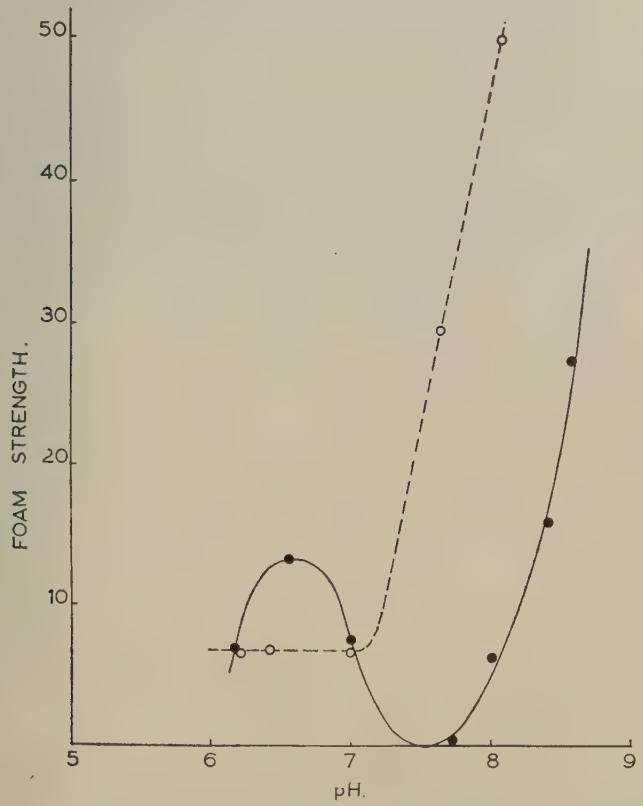


Fig. 5.—The effect of pH on foam strength of two samples of bovine saliva collected from the mouth.

collected and foamed under identical conditions were found to have an appreciable peak in the region of pH 6.0 6.5. Lyttelton (pers. comm.) by electrophoretic analysis has shewn that saliva collected as above has variable amounts of two major components. There is a possibility that this may be a significant animal factor in bloat and is being investigated further.

The effect of salt concentration on the salivary mucoprotein is complex and may also be a reflexion of the heterogeneous nature of this material.

Rumen Liquor

Effect of pH. Crude rumen liquor was collected 1 h after the commencement of feeding with red clover and cleared by centrifuging in the cold. The pH of aliquots was adjusted by the addition of acetic acid or sodium hydroxide solution. Foam strength (Fig. 3) had a distinct optimum at pH 5.4, and above pH 6.0 decreased rapidly. Dynamic foam stability similarly has a maximum in the same pH range but is not well defined. Other samples of rumen liquor had pH optima in the narrow range of 5.4 to 5.7.

Effect of Rumen Particulate Matter. In a large number of experiments during three years it has been found that the foaming properties of rumen liquor are greatly increased by high-speed centrifuging. Crude rumen liquor, in the foaming apparatus used, normally gives a coarse-bubble foam with poor foam strength. After centrifuging, the supernatant, provided conditions are suitable, may give a fine-bubble foam with high foam strength. This is demonstrated in the feeding experiment data in Table 3. The particulate matter, consisting of a mixture of plant cell debris, chloroplast fragments, bacteria, and protozoa, appears to have considerable anti-foaming properties, but alters the quality of the foams as shown by foam-strength measurements and appearance rather than the volume of foam produced.

Chloroplasts contain a very high concentration of lipids (Chibnall 1939; Neish 1939; Weeninck, unpubl. up to 37% of the dry weight, depending on the species. Scott (1955) has demonstrated that much of the lipid of the chloroplast occurs as a cloud of globules surrounding the chloroplast and attached to the parent body by transparent plasmodesmata. Such a structure would be expected to give chloroplasts high anti-foaming properties. It has been found (Mangan and Fraser, unpubl. that isolated chloroplasts have in fact appreciable anti-foaming properties. Fat-free chloroplasts, obtained by washing with acetone and ether, have no anti-foaming properties and have the opposite effect of stabilising foams. It seems probable that the anti-foaming agent in crude rumen liquor is chloroplasts or chloroplast fragments.

Effect of Feeding. The cows, with permanent rumen fistulae, were deprived of food for about 16 hours prior to the commencement of these experiments. The 0 h samples were withdrawn from the rumen, the animals given freshly cut red clover and further rumen samples

TABLE 3. FOAMING PROPERTIES OF RUMEN LIQUOR IN RELATION TO FEEDING

Experimental	Time (h)	pH	Foam Expansion (Vol. foam/Vol. soln)		Dynamic Foam Stability (% perfect stability)		Foam Strength (100 × Time/Distance)	
			Crude Rumen Liquor	Clear Rumen Liquor	Crude Rumen Liquor	Clear Rumen Liquor	Crude Rumen Liquor	Clear Rumen Liquor
Cow 293. Fed freshly cut red clover. Samples foamed with CO_2 at natural pH	0	6.77	2.82	3.23	42.2	48.9	0	0
	1	6.38	4.57	>6.63	68.9	89.0	0	0.1
	2	5.95	>6.63	>6.63	100	100	0.2	35.0
	4	5.99	5.00	5.00	73.4	75.6	0.1	0
	6	6.33	3.38	0.58	51.1	8.9	0.1	0
Cow 294. Fed freshly cut red clover. Samples foamed with CO_2 at natural pH	0	6.79	0.58	3.69	8.9	55.5	0	0
	1	6.33	>6.63	>6.63	100	89.0	0.2	8.3
	2	6.08	4.18	>6.63	63.3	100	0.9	39.5
	4	6.00	4.71	>6.63	71.1	100	0.3	16.1
	6	6.41	4.11	>6.63	62.2	100	0	0.9
Cow 294. Fed freshly cut red clover. All samples adjusted to pH 6.2 and foamed with CO_2	0	6.2	—	0.6	—	12.3	—	0
	0.5	6.2	—	>6.0	—	73.0	—	2.7
	1	6.2	—	>6.0	—	93.7	—	182
	2	6.2	—	>6.0	—	88.3	—	145
	4	6.2	—	0.4	—	9.9	—	0

taken at intervals. Feeding was continued for 2 h and the feed removed. From Table 3 it can be seen that the foaming properties of the 0 h rumen samples with both crude and clear preparations were very poor in all cases. As feeding proceeded, foaming properties increased rapidly to the point where feeding was discontinued and then decreased rapidly. With the crude rumen-liquor samples, foam strength was low, and the effect of feeding is seen only in foam expansion and dynamic foam stability. The foams were coarse in structure with considerable internal breakdown in evidence. In contrast the clear rumen liquor, except for the 0 h samples, gave fine-bubble foams with little or no internal breakdown. This is reflected in the greatly increased foam strength values.

The foams were generated with carbon dioxide at 37°c in order to maintain the pH relationships which occurred in the rumen at the time of sampling. Measurements of pH were taken immediately on removal of the samples from the animal and again after foaming. In all cases, except the 0 h samples, the two values were in close agreement. At 0 h the partial pressure of carbon dioxide in the rumen is low (Brodie 1945) and on foaming with carbon dioxide the pH of the samples drops appreciably.

In the third example in Table 3 (Cow 294) the pH values were not those occurring in the rumen, but all samplings were adjusted, by the addition of acetic acid, to a pH of 6.2, approximately the pH at which bloating is initiated. It may be concluded from these and numerous results not quoted that the foaming agent is released into the rumen liquor only while the animal is eating and also that it is rapidly metabolised by the rumen micro-organisms.

Discussion

Legume bloat in cattle is a complex disorder in which a number of factors must be at an optimum before bloating can occur. Although we are not aware of all the factors involved, it appears probable that the following general conditions would be important. First the active foaming agents must be released into solution in the rumen liquor, build up to a required concentration, and remain so for sufficient time for a foam to be formed. Secondly, conditions of pH, salt concentration, and perhaps other factors in the rumen liquor, must be suitable for stable-foam formation. Thirdly, sufficient gas must be generated by fermentation, and under some conditions of rumen pH from the bicarbonate of saliva, to generate the foam. Fourthly, anti-foaming agents from the herbage eaten must be in low enough concentration, or be sufficiently inactivated, to allow the foam to persist.

The most striking effect observed in this work is the marked dependence of foam strength on pH with the four systems studied. Red-clover saponin has an optimum at pH 4.5 to 5.0, red-clover cytoplasmic protein at pH 5.4 to 6.0, while salivary mucoprotein has maximum foam strength in the alkaline region of pH 7.5 upwards, but with a second peak in some samples at pH 6.0–6.5. Normally pH values found in the rumen liquor are in the range 6.0 to 7.0 with

most values around pH 6.5. Under the feeding regimen used in this laboratory, the pH of rumen liquor of cows fasted overnight is in the range 7.0 to 7.5, but when feeding on fresh clover commences the pH drops rapidly. Reid (unpublished work in this laboratory) has shown that under these conditions bloat can commence only at a pH of 6.3 or lower and, as the bloating condition develops, the pH drops further, with a minimum of about 5.7. Of the systems studied, cytoplasmic protein more nearly fits the pH requirements, and the actual foam strength/pH curve for clear rumen liquor is very similar to that of cytoplasmic protein at a concentration of 10 mg N/100 ml and a salt concentration of 0.1 N. It should be noted, however, that at higher protein and salt concentrations the pH optimum shifts towards 6.0.

It has been found (in preparation) that cytoplasmic protein is released rapidly into solution in the rumen liquor in quite large amounts while the animal is feeding and may rise from a low level to a concentration of 20 mg N/100 ml one hour after the commencement of feeding fresh red clover. As shown in the experimental sections, foaming of solutions of cytoplasmic protein are greatly affected by salt concentrations. Cation concentration in rumen liquor has been found to be in the range 0.1 to 0.2 M, predominantly sodium and potassium, but with appreciable amounts of calcium and magnesium and variable levels of ammonium. Salt concentration in this range with a cytoplasmic protein level of 20 mg N/100 ml is such as to allow a very strong and stable foam to be formed.

Examination of the foaming properties of rumen liquor by the methods employed in this investigation has demonstrated two points. (1) The foaming activity of rumen liquor increases while the animal is eating, and decreases, in most cases rapidly, after the cessation of feeding. This is consistent with the view that the foaming agent is preformed in the clover and released by chewing, or that it is secreted in the submaxillary or sublingual (residual) salivas, which are stimulated by chewing. For a bacterial product such as a polysaccharide slime (Hungate *et al.* 1955) to be responsible, it would be necessary to show that the rate of synthesis can be rapid enough to produce bloat in as little as 30 min and that when feed intake ceases the product disappears rapidly from the rumen liquor. Levels of cytoplasmic protein in the rumen liquor have been estimated (Mangan, unpubl.) and found to follow the foaming properties. (2) The anti-foaming properties of the particulate matter in the rumen liquor exert a marked effect and may largely mask the potential foaming properties of the clear rumen liquor. Wallace (1958) considers that, in feed-lot bloat, food particles stabilise the foam bubbles by adhering to the air/water interface. This effect has also been observed with small particles in foams from cows bloating on clover. De-fatted chloroplasts have been found to have foam-stabilising action but normal chloroplasts have the opposite effect. It appears that the particulate matter of rumen liquor may have a dual role, but in clover-fed animals the anti-foaming properties predominate.

In attempts to obtain a correlation between the foaming properties of rumen liquor and bloat, major difficulties were encountered.

From first principles, however, this is not unexpected, since surface-viscous compounds such as proteins are readily surface denatured. Once the material has been surface spread in the form of a foam it is converted to the insoluble denatured form. Thus when the foam is broken it cannot be foamed again under experimental conditions. This is normally observed when foaming solutions of cytoplasmic protein. Red-clover saponin does not become insoluble when its foams are collapsed but changes have occurred and the solution, if dilute, cannot be satisfactorily foamed again. If a cow is fed very potent red clover the animal may bloat after a small intake of about 20 lb. The rumen ingesta will be found to be a mass of foam and the liquid that can be drained from this foamy material will be found to have poor foaming properties. In effect, the greater part of the active material is in the form of an irreversible foam. In such cases it is necessary to obtain samples before foaming in the rumen has progressed too far. On the other hand, if the animal is fed only moderately potent clover the intake may be as high as 50 lb before foaming is excessive and the rumen liquid will show good foaming properties.

Although the optimum pH for foam strength with red-clover saponin and salivary mucoprotein is outside the range we are concerned with in bloat, these compounds do form foams at pH 6 and together with cytoplasmic protein may give foams with greater foam strength than either alone. In addition, some saliva samples have a secondary peak for foam strength at pH 6.0–6.5 which may be important. It may be concluded, however, that if the measurement of foam strength used in the present work has any correlation with the foam conditions in the rumen necessary for bloat, cytoplasmic protein would appear to be of major importance as a component of the foaming system.

Acknowledgements

I am grateful to Miss M. E. Brown and Mr. P. Vlieg for their technical assistance and to Mr. C. S. W. Reid, physiologist, for his ready co-operation in work with the experimental animals.

References

Brodie, S. 1945: "Bioenergetics and Growth". Reinhold Publishing Co., New York.

Chibnall, A. C. 1939: "Protein Metabolism in the Plant". Yale University Press.

Chibnall, A. C.; Rees, M. W.; Williams, E. F. 1943: *Biochem J. 37:* 354.

Clark, N. O. 1947: "Mechanically Produced Foams for Combating Petrol Fires". H.M. S.O., London.

Cumper, C. W. N. 1953: *Trans. Farad. Soc. 39:* 1360.

Ferguson, W. S.; Terry, R. A. 1955: *J. agric. Sci. 46:* 257.

Foulk, C. W.; Miller, J. N. 1931: *Industr. Engng. Chem. 23:* 1283.

Hungate, R. E.; Fletcher, D. W.; Dougherty, R. W.; Barrentine, B. F. 1955: *Appl. Microbiol. 3:* 161.

Johns, A. T. 1954: *N.Z. J. Sci. Tech. A36:* 289.

Lindahl, I. L.; Cook, A. C.; Davis, R. E.; Maclay, W .E. 1954: *Science 119:* 157.

Lyttleton, J. W. 1956: *Biochem. J. 64:* 70.

Mangan, J. L. 1958: *N.Z. J. agric. Res. 1:* 140.

Neish, A. C. 1939: *Biochem. J. 33:* 293, 300.

Perri, J. M.; Hazel, F. 1947: *J. phys. Chem. 51:* 661.

Reid, C. S. W.; Johns, A. T. 1957: *N.Z. J. Sci. Tech. A38:* 908.

Scott, F. M. 1955: *Amer. J. Bot. 42:* 475.

Wallace, V. 1958: *Proc. 4th Conf. on Rumen Function, Chicago.*

Walter, E. D.; Van Atta, G. R.; Thompson, C. R.; Maclay, W. E. 1954: *J. Amer. chem. Soc. 76:* 2271.

BLOAT IN CATTLE

XII. THE EFFECT OF ORALLY ADMINISTERED PENICILLIN ON BODY WEIGHT OF LACTATING COWS, ON MILK PRODUCTION, AND ON PRODUCTION AND COMPOSITION OF FAT

By A. T. Johns,[1] F. H. McDowall,[2] and W. A. McGillivray[3]

(*Received for publication, 29 August 1958*)

Summary

Procaine penicillin was given every third day to one cow of each of three monozygous twin pairs. The rates were 100,000 units per dose over a period of 15 days, 500,000 units per dose over a period of 12 days, and 200,000 units per dose over a period of 5 months. There was no evidence of transfer of penicillin to the milk. There was no adverse effect on the weights of the cows, on the yields of milk and butterfat, on fat and solids-not-fat contents of the milk, or on the iodine value, saponification value, softening point, Reichert value, carotene content, and vitamin A content of the butterfat. On this evidence it appears that penicillin may safely be used under New Zealand conditions for treatment and control of bloat at dose rates up to 500,000 units every third day.

Introduction

The effectiveness of the oral administration of aureomycin, terramycin, bacitracin, streptomycin, and penicillin, for the prevention of bloat in steers has been investigated by Barrentine *et al.* (1956). Of these antibiotics, penicillin was the only one which prevented bloat and which had no ill-effect when a single dose of 300 mg or less was given. In a preliminary test with a dose rate as high as one gramme of antibiotic, diarrhoea was severe in all cases.

Barrentine and his co-workers reviewed the work done up to 1956 on feeding penicillin to ruminants and noted that there is little information available on the effect of penicillin on rumen organisms. Numerous investigations have been made on the effect of the use of antibiotics in feeds for young farm animals, but prior to the finding that penicillin has bloat-preventive properties there has not been any reason to include them in feeds for adult dairy cattle. There are obvious dangers to be considered in the use of orally administered penicillin for bloat control:

(a) It could conceivably unduly depress the activity of the Gram-positive bacteria in the rumen, and so reduce production of some or all of the volatile fatty acids. (Experiments with fistulated animals have shown that penicillin does reduce the rate of volatile acid production and as a result there is a

[1] Plant Chemistry Division, Department of Scientific and Industrial Research, Palmerston North.
[2] The Dairy Research Institute (N.Z.), Palmerston North.
[3] Massey Agricultural College and The Dairy Research Institute (N.Z.), Palmerston North.

temporary accumulation of lactic acid (unpublished results)). This could lead to a temporary shortage of the acetate for fat synthesis, and so could possibly affect the production and properties of the butterfat.

(b) It could reduce microbial hydrogenation of the unsaturated pasture lipids (Shorland *et al.* 1957), leading to an increase in the degree of unsaturation of the butterfat.

(c) As a result of (b) the uptake of fat-soluble vitamins could possibly be altered through the different composition of the lipids in which they are dissolved.

(d) It could conceivably be absorbed into the bloodstream and thence into the milk, and so lead to secondary effects such as development in the udder of penicillin-resistant strains of mastitis organisms, and retardation of starter growth in cheese-making operations. Before oral administration of penicillin could be recommended for the control of bloat in dairy cows, evidence was required on the extent of the possible secondary effects of the treatment.

At the time of commencement of the work herein reported there was no relevant information available in the literature on the effects of administration of penicillin to dairy cows, but since then several papers on the subject have been published.

Emery *et al.* (1958), quoting from unpublished work, found that the inclusion of 400 mg of procaine penicillin in the ration often caused a marked depression of milk production for several days. They state: "This depression of milk production may be accompanied by loss of appetite and other symptoms, including discharge at the nostrils and hyperemia of the vaginal mucosa". These symptoms were noted, too, in one herd receiving only 100 mg procaine penicillin. Emery *et al.* (*loc. cit.*) compared also the monthly butteraft productions in 15 herds of cows given 100 mg of procaine penicillin either daily or every third day with the productions from 15 herds managed in the same manner but not given penicillin. The average of the 144 monthly butterfat production records for the cows of the treated herds was 38.5 lb and that of the 187 monthly records for the control herds was 38.7 lb. The difference, 0.2 lb, was not significant.

Murnane (pers. comm.) in Australia compared, over a period of five weeks, the daily milk yield and weekly fat tests of the milks from 8 cows given 400,000 units of penicillin daily for four weeks, with the corresponding data for 8 cows not treated with penicillin. There was no significant change in either milk yield or fat test of the milk as the result of the treatment. The only adverse effect of the treatment was a transient diarrhoea in some of the cows during the first few days of treatment.

Working with 36 steers grazed on lucerne, Johnson *et al.* (1958) found that body-weight gains of animals fed 75 mg penicillin daily were greater than for the controls.

The present paper gives the results of an investigation in which doses of 200–500 mg of procaine penicillin were given to milking cows. For the main portion of the investigation (Trials B and C, see below) monozygotic twins were used because of their well-known advantages for work on factors influencing milk yield and composition and butterfat properties (McDowall and Patchell 1958).

Experimental

There was a short trial (Trial A) for investigation of the effect of treatment with penicillin on the penicillin content of milk. The antibiotic, in the form of 100,000-unit lactose-based tablets* of procaine penicillin, was administered by means of a balling gun. One cow was given 100,000 units of penicillin after the evening milking and milk samples were taken at both the morning and evening milkings for the following two days. Later, three cows were treated with 500,000 units of penicillin after the evening milking, and a composite sample of the milks from the three cows was taken at both the following morning and evening milkings. Penicillin was estimated in the milk by the method of Whitehead and Cox (1958).

For the work on milk yield and composition, and on properties of butterfat, three pairs of two-year-old† twins were used. Unfortunately one of the treated cows withheld a portion of her milk at each milking, and so gradually dried herself off (an analysis of the milk yields for both control and treatment periods showed that this effect was not due to the treatment). The data for this twin pair therefore were not valid for the purpose in view. The cows of the two remaining twin pairs had calved within the two months preceding commencement of the trials, and during the trials were grazed on a mixed ryegrass/white-clover pasture. On the treatment days, one cow of each pair was given penicillin after the evening milking. There was both a short-period trial (Trial B) and a long-period trial (Trial C), as follows:

Trial B—

Period I 7-16 Oct., 56	Pre-dosing uniformity control period of 9 days, no treatment.
Period II 16-31 Oct., 56	Dosing with 200,000 units of penicillin every third day for 15 days.
Period III 31 Oct.- 11 Nov., 56	Dosing with 500,000 units of penicillin every third day for 12 days.
Period IV 10-27 Nov., 56	Post-dosing control period of 16 days, no treatment.

* Tablets for all the trials were kindly supplied by Glaxo Laboratories (N.Z.) Ltd., Palmerston North.

† For work of this type with monozygous twins it is preferable to use cows that have been at least one season in milk, but at the time when the present investigation was undertaken all the older twin pairs in the Dairy Research Institute herd were in use for other purposes.

Trial C—

Period I 2 Dec. 56-17 Feb., 57	Dosing with 200,000 units of penicillin every third day for 5 months, commencing 2 December 1956. Results considered as two consecutive periods.
Period II 18 Feb.-8 May, 57	

In Trial C the cows were weighed weekly, as a check on the effect of penicillin treatment on the general well-being of the animals.

In Trials B and C milk yields were measured daily. Composite samples of evening and morning milks from each cow were taken daily during Period I and the first week of Period II of Trial B and thereafter at intervals during the remainder of the trial. Samples were taken twice weekly during the whole of Trial C. The milks were analysed for fat and solids-not-fat contents.

At intervals, portions of the daily composite samples of milk were separated, and the creams were churned for preparation of butterfat samples as described by Dolby (1954). Refractive index of the butterfat was measured in an Abbé refractometer at 40°c, iodine and Reichert values were estimated by the British Standard Method (1952), softening points were determined by the falling ball method as described by Barnicoat (1944), and carotenoids and vitamin A alcohol and ester contents of the butterfats were estimated as described by Thompson *et al.* (1949).

RESULTS

Penicillin Assays on Milk (Trial A)

There was no evidence of any penicillin in the milks analysed. Since the sensitivity of the test of Whitehead and Cox (1958) is 0.1 unit of penicillin per ml of milk, it can be safely concluded that dosing of cows with procaine penicillin for the prevention and treatment of bloat will not result in transfer of significant amounts of penicillin to the milk. The question as to whether treatment with more soluble forms of the antibiotic would lead to appearance of appreciable quantities in the milk has not been investigated.

Health and Weight of the Cows

There was no observable effect of the dosing with penicillin on the health of the treated cows. This observation was supported by the day-to-day uniformity in yield of milk from these cows.

The difference in weights of the cows of the two twin pairs at the beginning, middle, and end of Trial C are shown in Table 1. There was no significant change in the comparative weights of the cows in each twin pair attributable to the treatment (compare the uniformity of the results for relative weights of monozygous twin cows kept under uniform conditions, McDowall and Patchell (1958).

Yield of Milk

The results for Trial B are given in Table 2. The yields of milk from the cows were of the order of 20–25 lb daily. The differences

TABLE 1. DIFFERENCE IN WEIGHTS OF COWS OF EACH TWIN PAIR AT BEGINNING, MIDDLE, AND END OF TRIAL C

Time of Weighing	Differences* in Weights (lb)	
	Cows 41, 42	Cows 59, 60
Beginning of trial (2 Dec.)	− 4	+15
Middle of trial (19 Feb.)	−15	+ 9
End of trial (5 May)	−20	+12

* Negative sign in this and subsequent tables indicates weight of treated cow lower than weight of control cow.

in yields between the control and treated cows of each twin pair remained substantially the same during the period of treatment, indicating that the dosing with antibiotic was not affecting the yield of milk.

TABLE 2. DIFFERENCE BETWEEN MEAN DAILY MILK YIELDS FOR COWS OF TWIN PAIRS, TRIAL B

Experimental period	Total No. of Days	No. of Sampling Days	Differences (lb) in Mean Daily Yield		
			Cows 41, 42	Cows 59, 60	Average
I	9	8	+3.0	−3.5	−0.2
II	15	7	+0.60	−4.1	−1.7
III	12	6	+1.9	−2.9	−0.5
IV	16	4	+0.9	−4.4	−1.7

The results for Trial C over the periods 2 December to 17 February, and 17 February to 8 May, are given in Table 3. Again there were no significant changes in the differences between the yields for the cows of each twin pair during the trial, and the differences during this trial were in the same direction for each twin pair as during the pre-treatment and post-treatment control periods of Trial B. This is in line with the findings of McDowall and Patchell (1958) in their uniformity trial on monozygous twins, viz, that differences in yield of milk from the cows of a twin pair tended to remain steady and in the same direction throughout a lactation, and it would indicate that there was no effect of penicillin treatment on the yield of milk in this trial.

Fat Test of Milk

Results for Trial B are given in the top section, and for Trial C in the bottom section, of Table 4. The changes in the differences are not outside the range of what could be expected for the cows of monozygous twin pairs (McDowall and Patchell 1958). Except in one

TABLE 3. AVERAGE DAILY YIELD OF MILK FROM COWS OF TWO TWIN PAIRS IN TRIAL C

Period	Average Daily Yield (lb)		Difference	Average Daily Yield (lb)		Difference	Mean Difference
	Cow 41	Cow 42		Cow 59	Cow 60		
2 Dec.-17 Feb.	18.5	19.6	+1.1	19.8	18.6	−1.2	0
18 Feb.-8 May	12.7	13.8	+1.1	16.1	14.1	−2.0	−0.5

TABLE 4. DIFFERENCE BETWEEN MEAN DAILY FAT TESTS OF MILK FOR COWS OF TWIN PAIRS, TRIALS B AND C

Experimental Period	Total No. of Days	No. of Sampling Days	Difference in Mean Daily Fat Tests of Milk (percent)		
			Cows 41, 42	Cows 59, 60	Mean
Trial B					
I	9	8	−0.19	−0.34	−0.26
II	15	8	−0.60	+0.09	−0.26
III	12	6	−0.33	+0.14	−0.09
IV	16	4	−0.13	−0.06	−0.09
Trial C					
I	84	12	+0.02	+0.15	+ .09
II	77	11	−0.10	+0.51	+ .20

TABLE 5. DIFFERENCE BETWEEN MEAN DAILY FAT YIELDS OF MILK FROM COWS OF TWIN PAIRS, TRIALS B AND C

Experimental Period	Total No. of Days	No. of Sampling Days	Difference in Mean Daily Fat Yield (lb)		
			Cows 41, 42	Cows 59, 60	Mean
Trial B					
I	9	8	−0.14	−0.26	−0.06
II	12	8	−0.08	−0.19	−0.14
III	15	6	−0.03	−0.08	−0.03
IV	16	4	−0.01	−0.19	−0.09
Trial C					
I	84	12	+0.056	−0.098	−0.021
II	77	11	+0.076	+0.009	+0.040

instance, the changes that did occur were in the direction of increase in relative yields for the cows treated with penicillin.

Fat Yield

Results for Trial B are given in the top section, and for Trial C in the bottom section, of Table 5. In Trial B there was no significant effect. In Trial C there was an apparent change, in the direction of an advantage for the treated animals, but the change was significant only at the 10% level.

Solids-not-fat Content of Milk and Solids-not-fat Yield

The results for Trial C are given in Table 6. The change in percentage solids-not-fat content of the milk, in the direction of favouring the treated animals, was significant at the 1% level. There was no significant change in solids-not-fat yield.

TABLE 6. Difference in Solids-not-fat Content of Milk and in Solids-not-fat Yields from Cows of Twin Pairs, Trial C

Period	Total No. of Days	No. of Sampling Days	Difference in Average Daily Result		
			Cows 41, 42	Cows 59, 60	Mean
Content in Milk (%)					
I	84	12	+0.02	+0.05	+0.03
II	77	11	+0.03	+0.41	+0.22
Yield (lb)					
I	84	12	+0.21	−0.25	−0.02
II	77	11	+0.13	−0.12	+0

Properties of Butterfat

The results for Trial B are shown in Fig. 1. The changes in the average differences between the results for the control and treated cows of the three twin pairs for all the values except Reichert value were not greater than the changes normally encountered in corresponding results for monozygous twin cows in a uniformity trial (cf. McDowall and Patchell 1958). Even with Reichert values the differences were small. They were in the direction of a decrease in the volatile acid content of the butterfat. If this difference was caused by the penicillin treatment it would indicate a slight impairment of acetate production in the rumen, as postulated above. That penicillin may have this effect was supported by the results of a short trial in which it was found that dosing with a million units produced a greater depression in Reichert values.

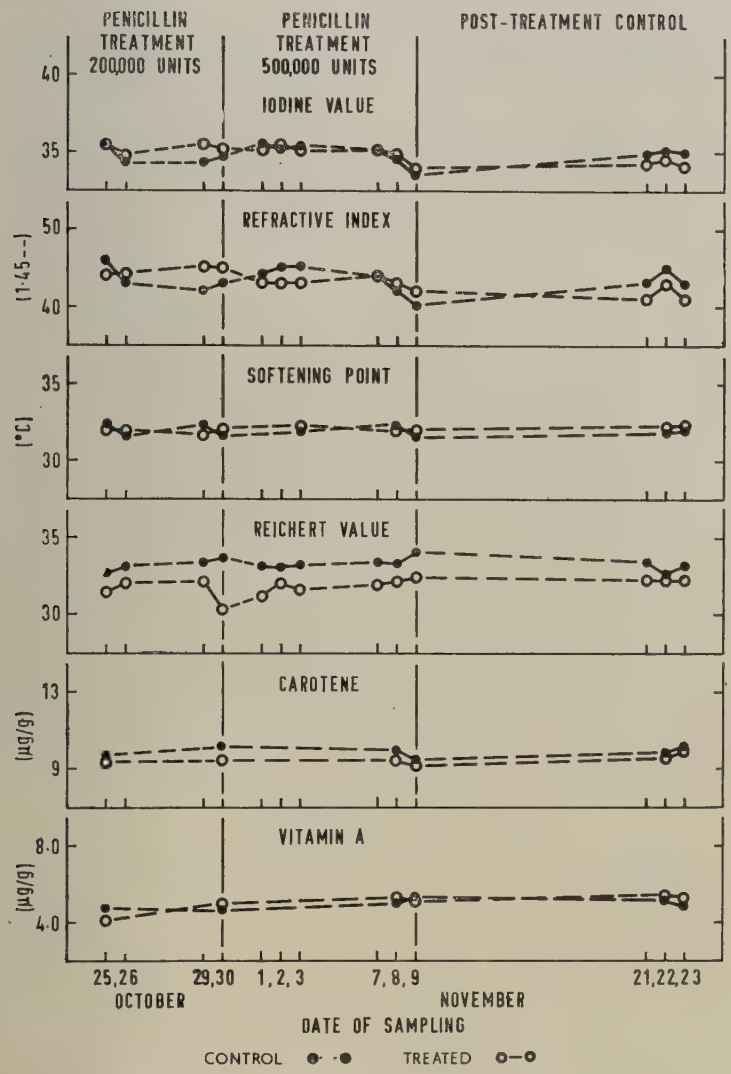


Fig. 1.—Butterfat from three control cows compared with butterfat from their treated monozoygous twin mates.

The differences observed, however, were of no practical importance.

Discussion

In the present trials there was, within the limits of the small number of cows used, no evidence that penicillin caused any adverse

effects with non-bloating animals. During numerous New Zealand field trials, only an occasional cow has shown either loss of appetite or marked depression in daily milk production. Whether this was due to the effect of treatment was not established. In these field trials the other observed effect has been an occasional marked increase in appetite when penicillin has been given during an outbreak of bloat, causing the cows to become so uncomfortably full that let-down of milk was inhibited. (This effect was not observed in the present trials with non-bloating animals, but when it does occur it can be overcome by rationing pasture with the electric fence.)

Reference has already been made to the observations by Emery *et al.* (1958) of some injurious effects of dosing with penicillin. Whether the differences between these findings and those of the present investigation were due to difference in the type of pasture grazed by the cows, causing production of a different balance of rumen bacteria, or to difference in breed of cow, is not established. It is worthy of note in this connection, however, that in the results of research investigations at different institutions there has been some lack of uniformity in the degree of effectiveness of orally administered penicillin as a means of control of bloat. Since, as is well known, severe bloat reduces intake (Johns 1954; Reid and Johns 1957) and hence milk production, it seems clear that where penicillin is found to give control of bloat its use when cows are grazing bloat-producing pastures will lead to greater milk production than would be obtained without the application of some effective prophylactic measure.

While it must be borne in mind that adverse effects have been reported by other workers, the indications of the present investigation, and of the field trials referred to above, are that the treatment would not cause any change of practical significance in the yields and composition of the milk and butterfat of a herd of cows under New Zealand conditions.

Acknowledgements

The authors are indebted to Mr. B. W. Taylor and the farm staff of The Dairy Research Institute (N.Z.) for the dosing of the cows and for the collection of milk and cream samples; to the staffs of the Chemistry Department of The Dairy Research Institute (N.Z.) and of the Biochemistry Department of Massey Agricultural College for assistance in the laboratory work, to Mr. G. A. Cox for the penicillin analyses, and to Mr. A. Glenday of the Department of Scientific and Industrial Research for assistance in the analysis of the results.

References

Barnicoat, C. R. 1944: *Analyst 54:* 75.

Barrentine, B. F.; Shawver, C. G.; Williams, L. W. 1956: *J. Animal Sci. 15:* 440.

British Standards Institution 1952: B.S. 769, 1952.

Dolby, R. M. 1954: *J. Dairy Res. 21:* 78.

Emery, R. S.; Smith, C. K.; Huffman, C. F. 1958: *Quart. Bull. Mich. agric. Exp. Sta. 40:* 460.

Johns, A. T. 1954: *N.Z. J. Sci. Tech. A36:* 289.

Johnson, R. H.; Brown, L. R.; Jacobson, N. L.; Allen, R. S.; Homeyer, P. G. 1958: *Report on Rumen Conference, Chicago 1957.* U.S. Dep. Agric., Washington.

McDowall, F. H.; Patchell, M. R. 1958: *J. Dairy Res. 29:* 159.

Reid, C. S. W.; Johns, A. T. 1957: *N.Z. J. Sci. Tech. A38:* 908.

Shorland, F. B.; Weenink, R. O.; Johns, A. T.; McDonald, I. R. C. 1957: *Biochem. J. 67:* 328.

Thompson, S. Y.; Ganguly, J.; Kon, S. K. 1949: *Brit. J. Nutr. 3:* 50.

Whitehead, H. R.; Cox G. A. 1958: *J. appl. Bact. 19:* 247.

A METHOD OF ESTIMATING THE BOTANICAL COMPOSITION OF THE DIET OF SHEEP

By Barbara H. Croker,* Extension Division, Department of Agriculture, Alexandra.

(*Received for publication, 11 August 1958*)

Summary

Fragments of plant cuticile found in the faeces are compared with a set of references prepared from the leaves of known plants. The cuticle patterns, as far as studied, are characteristic for each species and the fragments can be used to identify the plants grazed and an estimate made of the botanical composition of the diet. The results presented in this preliminary study are purely qualitative.

The advantage of this method is that it in no way interferes with the normal habits of the animal, and it may be used for any animals grazing on any type of pasture.

Introduction

Methods of Assessing the Diet of Grazing Animals

All grazing animals are selective in their diet. The availability and palatability of the herbage varies with the season and is different in each plant community. The various methods used to assess the diet of grazing animals have been reviewed by Tribe (1950). They include differential mowing techniques, metabolic cages, and harnesses for collecting the faeces. By these means the amounts of dry matter consumed, the protein content of the herbage eaten, and the amounts undigested can be estimated. However, no method of sampling a pasture has yet been found to give a plant sample guaranteed to represent the diet chosen by the animal. Recently, oesophageal and rumen fistulas have been used but there is some doubt as to whether these collect a representative sample of the diet, and the presence of the fistulas may interfere with the normal habits of the animal. These methods have been developed for use on experimental farms or in small pastures but are difficult or impossible to use for animals on extensive hill-country grazing.

Stapledon and Jones (1926), Fenton (1937), and other early workers followed the grazing animal and noted which species showed signs of grazing. Tribe (1950) suggested that deductions from botanical analysis before and after grazing combined with continuous direct observation of the animal was still the only method for open range land. On extensive hill country even this method has practical limitations both in the methods of botanical sampling and the ability of the observer to follow the animal. Recently Martin (1955) used the features on leaf cuticle to assess the botanical composition of the diet of sheep on heather grazing in Scotland. The basis of his method is that the fragmentary material in the faeces contains fibres, hairs,

* Present address: Mrs. J. M. Hercus, 2 Chancellor Street, Christchurch, N.1.

vascular tissue, and cuticle. Of these, the cuticle bears sufficient characteristic features to be identifiable as belonging to a specific plant.

The cuticle is a waxy material secreted over the epidermis of the leaf. When the growth of the tissues beneath is complete, the cuticle is firm, impermeable to water, and resistant to most chemical agents. It is a continuous layer which follows the contours of the cells. The outer wall of the epidermal cells may also be impregnated with cutin so that when the cuticle is either stripped off, or separated by maceration it bears an imprint of, and sometimes part of the cells of, the underlying epidermal layer. The structure and arrangement of the epidermal cells appear to be characteristic of the species, thus the pattern discernible on the cuticle is also characteristic.

The cuticle covering the actively growing tissue of the leaf meristem is very thin and readily disintegrates on maceration, so that only cuticle from mature tissues is likely to survive the processes of digestion. The thickness of the cuticle also varies from species to species; some have thin cuticles that do not survive chemical maceration, and are unlikely to survive digestion, so there will be no identifiable trace of these in the faeces. The only example encountered so far is *Anthoxanthum odoratum* (sweet vernal).

Methods Tried in the Tussock Grasslands of New Zealand

The tussock grasslands have been used for extensive grazing of sheep since soon after the arrival of the early settlers but we still do not know exactly which species are eaten by the sheep. Cockayne (1919) drew up a tentative list of relative palatability of tussock species after closely watching sheep on Conical Hill at Hanmer. He also recorded which plants showed signs of grazing in several other localities but these observations were complicated by the presence of rabbits, horses, and hares. He commented that "to ascertain that certain plants have been eaten. . . . The final decision must come from actually seeing a sheep feeding on a particular plant and proving from an examination of the plant itself that a portion has just been bitten off. This is no easy matter. In the majority of cases, even where a sheep has been grazing steadily for some time, it is difficult to find the exact spot where the sheep has been and equally, if not more, difficult to find the plant or plants which have been nibbled."

More recently J. M. Hercus (pers. comm.) has been using a method of botanical sampling. A one-foot-diameter ring thrown at random is used to sample the tussock grassland. All species present in the ring are rated according to the intensity to which each has been eaten. A series of observations has been made to show the seasonal pattern of grazing.

Both these methods, however, have practical limitations where the grazing intensity is one sheep to five to ten acres. The sheep range over large areas of steep hill country grazing a mosaic of tussock communities. A large number of species are present in any one community and changes in aspect, altitude, and soil moisture are reflected

in the floristic composition. The author's transfer to Alexandra afforded an opportunity to test the value of Martin's (1955) method of identifying the plants eaten by sheep on hill country.

Background to Experimental Material

The tussock grasslands are characterised by the slow-growing perennial species *Festuca novae-zelandiae, Poa colensoi,* and *Danthonia flavescens.* Although they are a distinct plant association, these grasslands are a mosaic of plant communities which occupy a wide range of habitats. The floristic composition varies with aspect, altitude, climate, and soil fertility, and also the invasion of exotic species. It is essentially a layered community in which the tussock leaves form a canopy over the inter-tussock vegetation of smaller grasses, herbs, and mosses. In a high-altitude snow-grass (*Danthonia flavescens*) community some 15–25 species are usually present, while at lower altitudes 35–60 species may be found in any one fescue (*Festuca novae-zelandiae*) community, including introduced grasses and clovers. In the South Island, over 210 indigenous and some 50 exotic species have been recorded from the tussock grasslands. It is thus of considerable interest to know what part the various species, native and introduced, play in the diet of the sheep at various times of the year.

Experimental Method

Preparation of Reference Slides

Fresh leaves were collected from 50 species growing in the field, and from these a set of permanent reference slides was made. The leaves were macerated in 50% nitric acid over a water bath, and the tissues washed in water to remove the acid. The cuticle was gently separated from the rest of the leaf using mounted needles and a camel-hair brush. It was then floated on to a slide and cleared of any remaining cellular material as far as possible with a fine jet of water and a brush. The slides were taken through the alcohol series from 15% to 95%, stained with 1% acid fuchsin, and permanently mounted in Euparal.

Diagrams were made of the pattern on the cuticle of each species. Both a projection method and a camera lucida attachment were used to give a magnification of about 430. The length and width of the cells were measured using an ocular micrometer, and notes were made on the arrangement and size of the stomata and any hairs or glandular cells present.

The slides were also photographed using a Leitz Panphot microscope and a 35 mm Leica camera with eyepiece attachment. Half-plate enlargements of these photographs give a magnification of 160 for the low-power and about 650 for the high-power views. All cell measurements were checked on these photographs.

The permanent slides, photographs, and diagrams are used as a set of references for the identification of cuticle fragments found in the sheep faeces.

Analysis of Faecal Samples

For the preliminary testing of the method, faecal samples were collected from two types of tussock grassland:

(1) At Glendhu Bay, a fescue community in a 35-inch-rainfall belt where a mixture of native and introduced species formed a good ground cover, and the block had been topdressed and oversown with grasses and clovers.

TABLE 1. NUMBERS OF CUTICLE FRAGMENTS OF EACH SPECIES IN FAECAL SAMPLES COLLECTED AT OPHIR, 2 DECEMBER 1957

Species	Samples								Total
	1	2	3	4	5	6	7	8	
Danthonia spp.	27	33	24	25	34	26	24	34	227
Agrostis tenuis	26	19	20	20	21	28	22	20	176
Carex sp.	12	13	11	10	12	3	2	8	71
*Flatweeds	20	19	18	22	27	19	15	26	166
Leontodon hispidus	1	3	2	3	2		3	2	16
Hypochaeris radicata	3	1		2	1	1	3		11
Crepis capillaris	4	8	3	3	4	7	6	4	39
Growing points (dicot.)	5	2	13	14	12	6	17	16	85
Smooth cuticle	29	36	31	33	23	26	24	36	238
†Species 'A'	20	24	26	20	19	24	25	22	180
Festuca rubra	10	7	11	3	8	11	6	7	63
Poa colensoi	7	4	6	8	8	6	6	6	51
Stellaria gracilenta	10	9	13	1	3	5	8	4	53
Poa pratensis	7	2	2	7	7	9	3		37
Vulpia dertonensis	2	4	5	7	3	5	3	4	33
Marrubium vulgare	3	6	1	5	2	7	7		31
Dactylis glomerata	1	?	1	4	3	3	9	8	29
Arrhenatherum elatius	6	2	1	1	1	4	6	1	22
Cerastium glomeratum	1		3	2	3		3		12
Bromus mollis	2		1	1	3	1	1		9
Holcus lanatus	3	2	1		2				8
Cirsium lanceolatum				3			3		6
Reseda luteola	1		1	1		4			7
Rumex acetosella		3	1				1		5
Trifolium arvense						1		1	2
Verbascum virgatum		1	1						2
Epilobium sp.					1		1		2
Festuca novae-zelandiae							2		2
Geranium sessiliflorum						1			1
Plantago lanceolata			1						1
Unknown		2	3	5	1	3		1	15
	200	200	200	200	200	200	200	200	1600

No fragments were found of *Erodium cicutarium, Trifolium campestre,* or *Raoulia lutescens.*

The cuticle patterns of *Bromus tectorum, Hordeum murinum,* and *Poa lindsayi* were not known.

* Flatweeds: this group includes cuticle fragments from rosette plants but they bore insufficient characters to determine the exact species.

† Species 'A' a grass cuticle very similar to *Aira caryophyllea.*

TABLE 2. NUMBERS OF CUTICLE FRAGMENTS OF EACH SPECIES IN FAECAL SAMPLES COLLECTED AT GLENDHU BAY, 21 NOVEMBER 1957

Species	Samples								Total
	1	2	3	4	5	6	7	8	
Danthonia spp.	13	24	8	15	24	18	19	25	146
Agrostis tenuis	15	16	26	14	11	17	9	22	130
Festuca rubra	7	12	6	32	16	12	11	9	105
Agropyron scabrum	7	11	17	18	5	9	18	17	102
Carex sp.	8	13	9	9	9	14	12	12	86
Smooth cuticle	52	41	53	40	40	45	51	44	366
*Species 'A'	35	29	27	37	42	38	35	28	271
†Flatweeds	13	8	12	8	8	15	10	16	90
Leontodon hispidus			2		2				4
Hypochaeris radicata	2	2	2						6
Crepis capillaris	1		3	1	5		1	3	14
Trifolium repens	16	11	5	6	5	7	6	9	65
Poa colensoi	10	7	10	5	9	4	4	5	54
‡Grass 'C'	4	1	7	5	5	7	4	3	36
Trifolium pratense	7	5		1	3	3	3		22
Luzula campestris	2	3	1		5	5	4	2	22
Celmisia gracilenta		3	2	2	3	3	4	3	20
Leucopogon fraseri	3	1	4	1	4	2	3	1	19
Cerastium glomeratum	1	5	2	1	1				10
Stellaria gracilenta	2	5							7
Blechnum penna-marina	1	1	2	1	1		1		7
Dactylis glomerata		?		2	1	1	4		8
Dichelachne crinita	1	2							3
§*Cynosurus cristatus*				1	1				2
Vulpia dertonensis	?						1	1	2
Rumex acetosella			2						2
Pimelea prostrata				1					1
Juncus sp.		?			?				
	200	200	200	200	200	200	200	200	1600

No fragments were found of: *Festuca novae-zelandiae, Holcus lanatus, Raoulia subsericea, Acaena pilosa, Prunella vulgaris, Helichrysum bellidioides, Wahlenbergia albomarginata,* or *Epilobium* spp.

Species present in the sward but cuticle patterns not known: *Deyeuxia avenoides, Hieracium pilosella, Pteridium esculentum, Gaultheria depressa, Microtis unifolia, Gnaphalium collinum,* and *Gentiana corymbifera.*

* Species 'A' a grass cuticle very similar to *Aira caryophyllea.*

† Flatweeds: this group includes fragments from rosette plants but cuticles bore insufficient characters to determine the exact species.

‡ Grass 'C' resembled the cuticle of *Dichelachne crinita* but lacked the distinctive hairs.

§ *Cynosurus cristatus* found as cuticle in the faeces, then a later visit to the area confirmed the presence of isolated plants.

(2) At Ophir, typical depleted country with a north-west aspect and 16-inch rainfall. Control of the rabbits had resulted in some improvement in the plant cover, but vegetation was still an open association of annual grasses and weed species with much bare ground. There were isolated clumps of cocksfoot

and tall oat grass which had spread through the fence from the Department of Agriculture's experimental area.

Eight samples were collected at random from each locality. As far as could be determined by observation, each sample was the droppings from one animal. A species list was compiled for the area together with notes as to which species showed signs of grazing.

TABLE 3. AN EXAMPLE OF THE SEPARATION OF SPECIES OF SIMILAR CELL PATTERN

A. Grasses

I. Cell walls straight
- (i) cells long, 250–400 μ × 20 μ—*Arrhenatherum elatius*
- (ii) cells smaller, 260 μ, with rows of stout, hook-like hairs—*Dichelachne crinita*

II. Rows of cells with stomata alternating with rows of cells with hairs.
- (i) cells large, walls straight, 150–230 μ × 30 μ, large hairs 200 μ, small hairs 40 μ—*Poa pratensis*
- (ii) cells large, 155 × 30 μ, few very long hairs, 700–950 μ—*Bromus mollis*
- (iii) cells 105–120 μ, walls finely corrugated, hairs abaxial, numerous, variable—*Festuca novae-zelandiae*
- (iv) cells small, narrowing at ends, 150 × 18–20 μ, hairs short, 20–30 μ—*Festuca rubra*

III. Cells conspicuously narrower at the end walls.
- (i) cells broad and short, 140 × 30 μ, hairs stout and hook-like, 35 μ—*Agrostis tenuis*
- (ii) cells long, hairs numerous, long and variable, 51–80 μ and 186–280 μ—*Holcus lanatus*

B. Herbs

I. Cells irregular in shape, many sided, like jig-saw puzzle pieces.
- (i) cells large, 79–105 × 25–40 μ, stomata large—*Blechnum penna-marina*
- (ii) cells large, 70–90 μ × 30–60 μ, hairs very large, multicellular and jointed, break at joints—*Cerastium glomeratum*
- (iii) cell walls less angled, stomata appear sunken—*Epilobium species*
- (iv) cell walls have sharp corners with small inward projections, unicellular hairs—*Geranium sessiliflorum*
- (v) cells small, 20–50 μ, hairs multicellular, appear to have glandular tips—*Crepis capillaris*
- (vi) cells small, large groups of glandular cells, fragments very small—*Cotula squalida*

II. Cells rectangular, 4–6 sided, not angled as above group.
- (i) cells small, 16–50 μ, triple hairs—*Marrubium vulgare*
- (ii) cells larger, 30–56 μ, hairs jointed—*Verbascum virgatum*
- (iii) cell outline angular, very thick hairs on the veins—*Erodium cicutarium*
- (iv) cells elongated parallel with veins, scattered thick hairs with very narrow central channel—*Plantago lanceolata*
- (v) cells, 40–80 μ × 20–40 μ, outline rounded, very long thin unicellular hairs, 350–440 μ—*Rosa eglanteria*

The faecal samples were diluted with an equivalent volume of water and sub-sampled. A thin film of material was spread between two slides and 50 identifications were made on each of four slides making a total of 200 identifications per sample.

The action of the digestive juices darkens the cellular tissues and imparts a light brown stain to the transparent cuticle, making the cell outlines and other features distinguishable. Thus no special treatment or staining of the faecal material is necessary. Samples may be preserved with formalin-acetic-alcohol if required, and examined at a later date. Fragments of cuticle between 0.1 mm and 1 mm usually have enough characteristic features to enable them to be identified as belonging to a specific plant. Larger fragments usually have too many cells and fibres still attached making them opaque and difficult to identify. In most cases it is possible to make specific identifications of the fragments under a magnification of 100 to 150.

RESULTS

Two hundred identifications were made for each of the eight samples collected from the two areas. The counts are presented in Tables 1 and 2. The objective was to find out which species were being grazed at that particular time and the results are not intended to be a quantitative estimate. No count was made of large undigested fragments.

DISCUSSION

For this study, reference slides were made from 50 species. The epidermal patterns of many of them were instantly recognisable, for example Figs. 1-4, *Aciphylla colensoi, Geranium sessiliflorum, Danthonia buchanani,* and *Festuca arundinacea.* Others were similar in pattern but differed in cell size, the size and distribution of stomata, and the presence and size of hairs. A key (Table 3) was compiled to facilitate the identification of some of these species. An example of difference in cell size and the presence of stomata is seen in *Rosa eglanteria* and *Leontodon hispidus* (Figs. 5, 6). Among the grasses the abaxial sur-

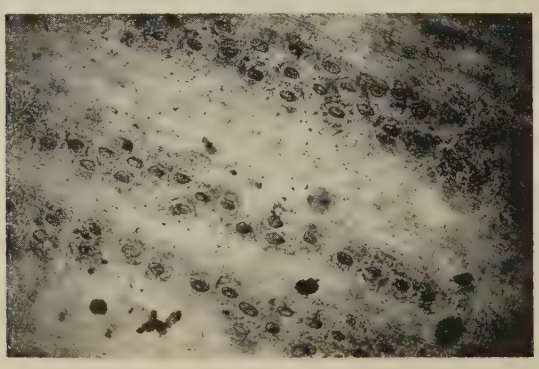


Fig. 1.—*Aciphylla colensoi*: distinctive bands of isodiametric cells and bands of sunken stomata, × 80

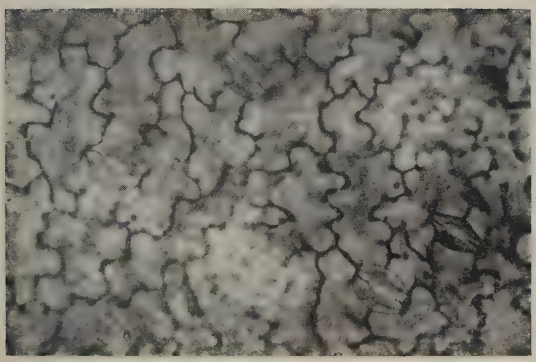


Fig. 2.—*Geranium sessiliflorum*: irregular cells with projections of thickening at the corners; numerous stomata. × 325.

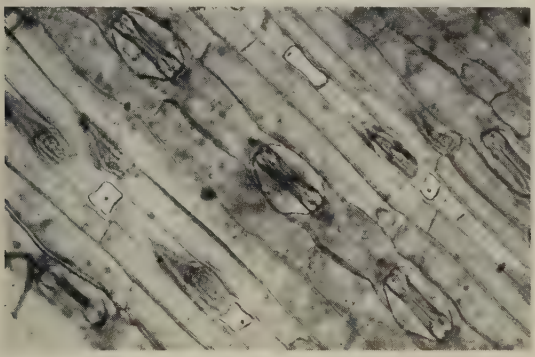


Fig. 3.—*Danthonia buchanani*: cells apparently smooth walled; numerous large stomata; characteristic opaline deposits. × 325.

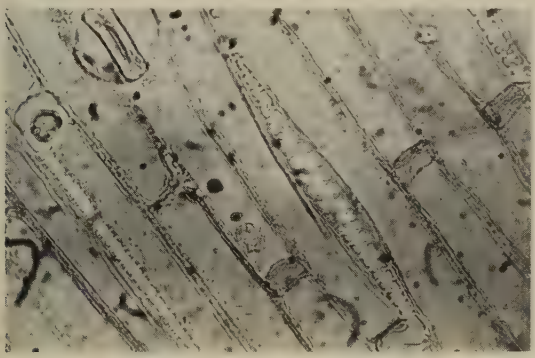


Fig. 4.—*Festuca arundinacea*: corrugated pattern of the cell wall very noticeable where cells pull apart. × 325.

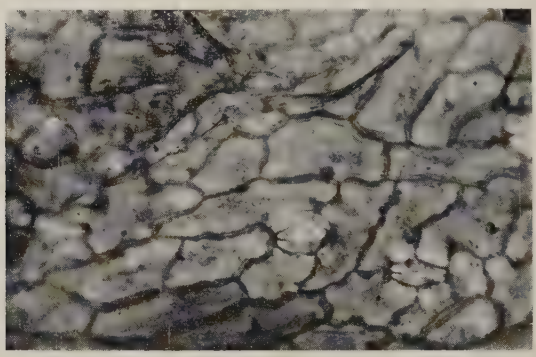


Fig. 5.—*Leontodon hispidus:* irregular cells 30–50 × 15–30 μ; epidermal cells extend over guard cells of stomata. × 325.

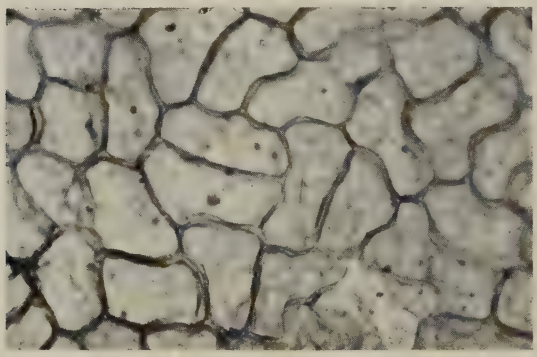


Fig. 6.—*Rosa eglanteria*: cells large and smooth in outline; no stomata on this surface of the leaf. × 325.

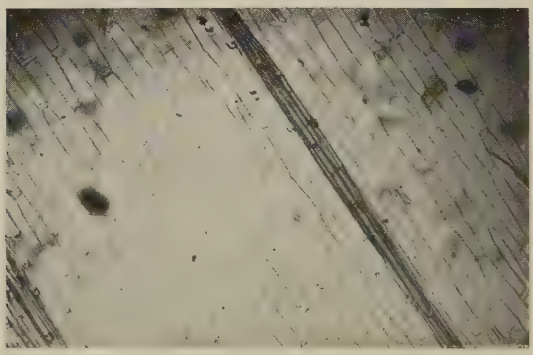


Fig. 7.—*Festuca arundinacea*: large cells; one row of stomata each side of vein. × 80.

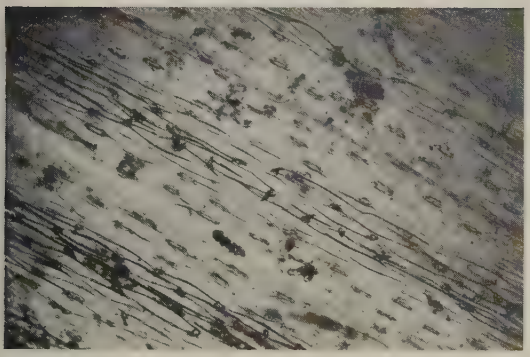


Fig. 8.—*Festuca rubra*: small cells; two rows of stomata each side of the veins and marked bands of small hairs. × 80.

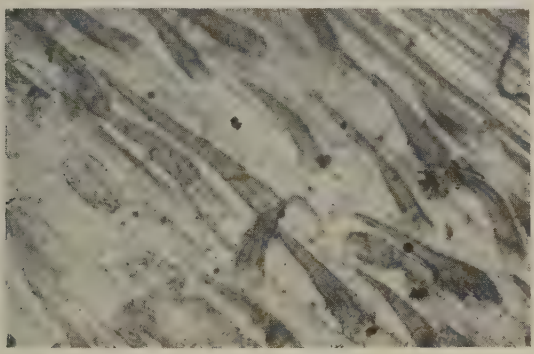


Fig. 9.—*Festuca novae-zelandiae*: same pattern as *F. rubra* (Fig. 8), but the stiff hairs obscure the cell outline. × 325.

Fig. 10.—*Medicago sativa*: long (850–940 μ), stiff hairs cause the cuticle to wrinkle, and distort the cell outline. × 80.

Fig. 11.—Hairs of herbaceous species: a. *Medicago sativa, b. Cerastium glomeratum,* c. *Plantago lanceolata,* d. *Crepis capillaris,* e. *Rosa eglanteria,* f. *Marrubuim vulgare,* g. *Geranium sessiliflorum,* h. *Verbascum virgatum.*

face of the fescue species differs in the size of both the cells and the hairs as in *Festuca arundinacea* and *F. rubra* (Figs. 7, 8).

Similarly the three species of *Danthonia* examined were alike in pattern but are distinguishable by the arrangement of the stomata and hairs. *D. buchanani* has fewer hairs than *D. semiannularis* and the hairs, 32–60 μ are smaller than in *D. pilosa;* it has larger stomata than *D. pilosa* and the stomata are closer together than in *D. semiannularis. D. pilosa* has two sizes of hairs, the larger are 120–155 μ and the smaller 28–35 μ. *D. semiannularis* has the largest cells, 110–130 μ $\times$ 10 μ. However no attempt was made to separate them in the sample counts.

The herbaceous species *Marrubium vulgare, Verbascum virgatum, Plantago lanceolata,* and *Hypochaeris radicata,* and also *Cerastium glomeratum, Geranium sessiliflorum,* and *Crepis capillaris,* are some-

what similar in cell pattern but each possesses distinctive hairs which are sufficient evidence of the presence of cuticle fragments of that species (Fig. 11). A dense covering of short stiff hairs makes it difficult to discern the exact cell pattern beneath, as in *Poa colensoi*, and to some extent in *Festuca novae-zelandiae* (Fig. 9). Long stiff hairs often make the cuticle curl and wrinkle. This is most marked in lucerne (Fig. 10). In these cases, however, the hairs were often sufficiently distinctive to enable an identification to be made. The only species in this study for which a cuticle pattern could not be obtained was *Anthoxanthum odoratum*.

The preliminary testing was done in two forms of the complex and variable community known as fescue-tussock grassland. A very large number of species were present and the first step was to find out whether this method of cuticle analysis would determine what species the sheep were eating at the time the samples were collected. No attempt was made to put it on a quantitative basis. The results obtained indicate that the method is a promising one and further work is being done to examine the quantitative aspect.

Interesting features of Tables 1 and 2 are the small amount of variation between the samples and the fact that the plants fall into several groups. The first group is represented by ten or more cuticle fragments per sample, the second by a few fragments in each and the third group is only occasionally represented. It is tempting to conclude that the first group represents those plants always grazed at that time of the year. The second group could then be regarded as those plants occasionally grazed, or those preferred but occurring only in small quantities in the pasture. This last was a noticeable feature at Ophir where only a few plants of *Dactylis glomerata* and *Arrhenatherum elatius* occurred along the fence line but all had been hard grazed. The plants that are represented by occasional fragments are perhaps eaten accidentally with other species. At Glendhu Bay *Dactylis glomerata* and *Cynosurus cristatus* are in this group but only isolated plants of these were found among the tussocks.

Two further groups must also be considered. *Anthoxanthum odoratum* was present in both areas but it has a thin cuticle that disintegrates during maceration and there is apparently no trace in the faeces. The second group contains the unknown cuticle patterns. The unknown "smooth cuticle" occurs in both sets of samples. Further work with fresh leaves has so far failed to give a similar transparent cuticle with no discernible features on it. It seems possible that it may come from flowering stems. Species "A" is probably *Aira caryophyllea* but the reference slide for this species was not prepared until after these counts were made. This grass was common in both areas. The cuticle called "grass C" bore few distinguishing features. Of the known patterns it most resembled *Dichelachne crinita* but lacked the distinctive hairs. In the Ophir samples there were a group of unknown fragments but no reference slides had been prepared for *Hordeum murinum*, *Bromus tectorum*, or *Poa lindsayi*. At Glendhu Bay there were also several species for which the cuticle patterns were

not known, but only two of these, *Hieracium pilosella* and *Deyeuxia avenoides,* are known to be eaten.

From his observations, Cockayne concluded that in general *Hypochaeris radicata, Crepis capillaris* and *Holcus lanatus* were eaten freely before any other grasses and herbs, followed by *Danthonia* species, *Poa colensoi, Dactylis glomerata,* and *Poa pratensis.* In the present study, fragments of *Danthonia* species were common in both sets of samples, *Poa colensoi* occurred in moderate numbers and the flat-weeds, as a group, including the separate counts for *Hypochaeris* and *Crepis*, formed a fair proportion of the fragments in both sets of samples. It is particularly interesting to note that at Ophir, where the vegetation is sparse, small growing points of herbaceous plants were often found more or less intact in the faeces suggesting close grazing of these species. Although there were only a few cuticle fragments of *Dactylis glomerata*, it occurred only as isolated plants in both areas and was well eaten. The same was true of *Poa pratensis* at Ophir.

The present results also confirm those obtained by Hercus in his observations, particularly for those plants in the first group in the tables.

Conclusions

The identification of the cuticle fragments present in the faeces seems a promising method of gaining information on the botanical composition of the diet of sheep. The method would be applicable to any animals grazing any type of vegetation. The results are of most value when considered in conjunction with a botanical analysis of the sward.

The advantages of this method are that it does not interfere with the normal habits of the animal as may occur with the use of harnesses and fistulas, and, as it imposes no restriction on the movement of the animals, it is of particular value where they range extensively over mixed communities such as the tussock grasslands.

This method may appear laborious, but is no more so than the pollen anaylsis used for dating peat deposits. Both methods depend on the preparation of and familiarity with a series of reference slides and diagrams made from known species.

Acknowledgements

I wish to express my gratitude to Professor G. T. S. Baylis for the use of the facilities and equipment in the Department of Botany, University of Otago, and to Dr. Brenda Slade for her help with the photography. I am also indebted to the Alexandra District High School for the loan of their microscope.

References

Cockayne L. 1919: An Economic Investigation of the Montane Tussock Grasslands of New Zealand. II. Relative Palatability for Sheep of the Various Pasture Plants. *N.Z. J. Agric. 27:* 321-31.

Fenton, E. W. 1937: The Influence of Sheep on the Vegetation of Hill Grazings in Scotland. *J. Ecol. 25:* 424-30.

Martin, D. J. 1955: Features on Plant Cuticle. An Aid to the Analysis of the Natural Diet of Grazing Animals with Especial Reference to Scottish Hill Sheep. *Trans. bot. Soc. Edinb. 36:* 278-88.

Stapledon, R. G.; Jones, M. G. 1926: The Sheep as a Grazing Animal and as an Instrument for Estimating the Productivity of Pastures. *Bull. Welsh. Pl. Breed. Sta., Ser. H, 5:* 42-53.

Tribe, D. E. 1950: The Composition of a Sheep's Natural Diet. *J. Brit. Grassl. Soc. 5:* 81-91.

Cercospora LEAF SPOT ON RHODODENDRON (*Cercospora handelii* Bubak)

By G. W. F. Maris McArthur, Horticulture Division, Department of Agriculture, Wellington.

(*Received for publication, 9 July 1958*)

Summary

A fungal disease on *Rhododendron,* not previously recorded in New Zealand was found in New Plymouth and Palmerston North. The fungus was identified as *Cercospora handelii* Bubak. It causes dark-brown angular spots on both sides of the leaves. The lower foliage of young stock is most susceptible. Control of the disease is discussed.

Introduction

In October 1957, specimens of *Rhododendron* leaves with brown angular spots on both sides were received from nurseries in New Plymouth and Palmerston North. The *Rhododendron* plants had been imported from Holland and were growing in quarantine areas of the nurseries. In New Plymouth, 20 plants were infected; in Palmerston North, 700 out of 1000. Shortly afterwards, in December 1957 and January 1958, additional specimens were received from two other nurseries in New Plymouth, where several varieties of *Rhododendron* were infected. The extent of infected plants was mostly very high, up to 100%.

The leaves and in a few cases the petioles were found to be infected with a fungus, identified as *Cercospora handelii* Bubak (*Cercospora rhododendri* Marchal et Verpl.), a disease which has not previously been recorded in New Zealand.

Symptoms

Angular dark brown spots on both sides of the leaves, varying in diameter from 3 to 20 mm. Separate spots may join together and then the greater part of leaves may become necrotic, especially round the borders. In a few cases the petioles were infected too. On some varieties of *Rhododendron* the centres of the spots were silvery white, owing to lifting of the epidermis. The disease seems to be most severe on the lower foliage of young stock. Under moist conditions a greenish-brown down can be seen on the spots, on both sides of the leaves, due to the fructifications of the fungus.

The Fungus

The greenish down on the spots consists of tufts of conidiophores emerging, through the stomatal openings, from a stroma, a pseudo-parenchymatous mass of cells that lies beneath the epidermis.

Conidiophores: subhyaline, in mass green-brown, not branched; straight or slightly curved, non-septate, 2–4 × 30–135 μ, each bearing one conidium at the apex.

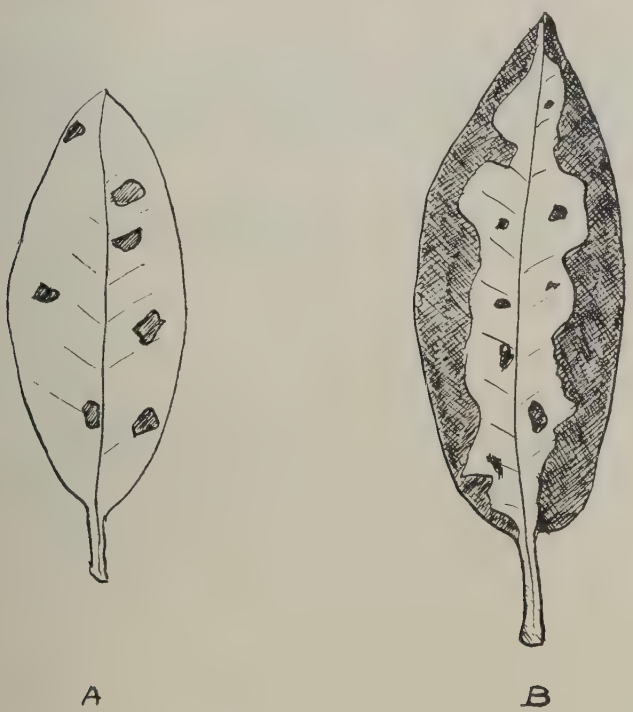


Fig. 1.—*Rhododendron leaves,* infected with *Cercospora handelii.* A. Slightly. B. Severely.

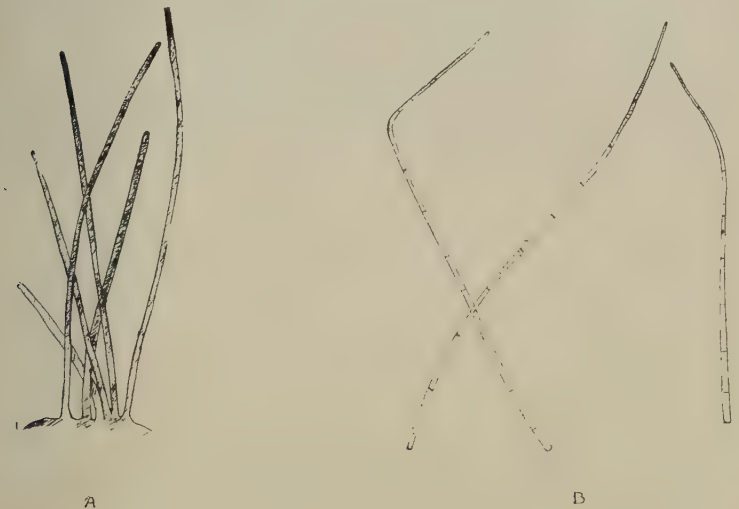


Fig. 2.—*Cercospora handelii* from *Rhododendron.* A. Conidiophores. B. Conidia.

Conidia: they developed readily on infected leaves when kept under damp conditions for 3 days. Subhyaline, in mass green-brown, linear to obclavate (widest near the base), more or less sharply curved, or undulate, multiseptate, 6–14 septa, 1,5–3,5 × 30–155 μ.

Hosts

Several varieties of *Rhododendron* were found to be affected: Purple Splendour, Earl of Athlone, Fatuosum, Blue Peter, Peter Koster, Prof. Hugo de Vries, and some unknown varieties.

Distribution

U.S.A., Holland, Belgium, Czechoslovakia, Southern Russia, Japan.

Control

It is important to remove the infected leaves. After that the plants should be sprayed with a fungicide. Control measures are still being investigated in Holland. In America a regular spraying with Bordeaux mixture during the growing season is recommended, while in Holland a mercury spray is suggested. In New Zealand both of these treatments were tried. Bordeaux has the disadvantage of being only protective and requiring many spray applications during the growing season, while mercury usually gives spray-damage.

In Palmerston North a spray with phenyl mercury chloride (wettable powder) at the rate of 2 lb per 100 gal water was applied. This gave spray damage, as could be expected; however the new growth was vigorous and clean.

The result of Bordeaux spraying in New Plymouth was satisfactory, and gave no spray damage. The disease did not spread on the older leaves after spraying, and the new growths were healthy.

However, a definite answer cannot yet be given, because the young leaves are usually not very susceptible to the disease. From the results obtained to date it appears that the best method of control is to remove and burn affected leaves and spray the plants with Bordeaux 6:8:100 at 14-day intervals.

Remarks

Spots on *Rhododendron* leaves, caused by *Cercospora handelii* may be confused by those caused by *Pestalotia macrotricha*. On some varieties they look rather similar. However, *P. macrotricha* is a secondary parasite. It is found in many cases where the plants are weakened by other causes, unfavourable conditions such as strong winds or parasites.

On many leaves infected with *C. handelii*, pycnidia of *P. macrotricha* occurred as well. The fructifications of this fungus can easily be distinguished from those of *Cercospora*. Under damp conditions long tendrils, consisting of the characteristic *Pestalotia* conidia, ooze out of the pycnidia.

Acknowledgements

I am grateful to Messrs. I. Mayo and T. N. Flint, Plant Quarantine Officers in New Plymouth and Palmerston North, who observed the disease in the field, collected specimens, and informed me of the results of the treatments.

References

Chupp, C. 1953: "A Monograph of the Fungus Genus *Cercospora*". New York, Ithaca.

Davies, S. H.; Hamilton, C. C. 1955: Diseases and Insect Pests of *Rhododendron* and *Azalea*. *New Jersey agric. Exp. Sta. Circ. 571.*

Director of Agriculture, The Hague (Ed.) 1958: *Tuinbouwgids, 1958.*

Marchal, E.; Verplancke, G. 1926: Champignons Parasites Nouveaux pour la Flore Belge, Observé de 1919 à 1925. *Bull. Soc. Bot. Belg. n.s. 9, 59:* 19-25.

White, R. P. 1933: The Insects and Diseases of *Rhododendron* and *Azalea*. *J. econ. Ent. 26:* 631.

ROTOMAHANA "MUD" NOT COBALT DEFICIENT

By I. J. Cunningham,† C. R. Taylor,* and A. G. W. Stevens*

(*Received for publication, 23 July 1958*)

Summary

Animal feeding experiments have shown that cobalt deficiency does not occur on Rotomahana sandy loam, which is a fertile volcanic soil formed from material discharged in the Tarawera eruption.

Introduction

Rotomahana sandy loam is the soil formed from the material discharged from craters in the lowland and to the south-west of Mt. Tarawera during the later stages of the Tarawera eruption of 1886. An area of some thirty-thousand acres was covered by varying depths of Rotomahana "mud" and it is this "mud", on which a thin rudimentary humus soil has developed, which responds well to phosphate fertilisers and is capable of carrying good pastures of ryegrass and white clover, suitable for farming.

Areas with a shallower cover of "mud" have been sucessfully farmed for 40–50 years, but the deeper deposits have in the main been developed and brought into production by the Department of Lands and Survey only during the last decade and handed over to settlers in the last 4 or 5 years. The chief reasons why the shallower "mud" soils were the first to be farmed are that they were easy of access and that prior to the eruption they were already cleared of forest.

In differentiating between the shallow and deep deposits of "mud" it is important to make clear that, whereas cultivation of the shallow sandy loam soils frequently resulted in mixing of the "mud" with the humus soil lying immediately below, there was no such amelioration of the thicker "mud" deposits. In fact, cultivation of the latter soils resulted in the thin surface layer of humus which had developed since the eruption of 1886 being buried so that the seed bed for pasture establishment was essentially raw "mud".

No special problems of stock health were disclosed on deep Rotomahana sandy loam in the earlier stages of agricultural development by the Department of Lands and Survey while sheep and run cattle were carried; nor have there been serious problems on large areas where this type of farming is still carried on. On the other hand some of the later subdivisions have been made to carry dairy stock and have been used for dairying within a very few years of first sowing down the pastures. On some of these areas there have been distinct problems of animal health. The most notable symptoms were debilitating spring scours similar to peat scours, and unthriftiness or deaths in young stock.

† Animal Research Station, Department of Agriculture, Wallaceville.
* Department of Agriculture, Rotorua.

A characteristic of pastures grown on deep Rotomahana sandy loam is that they have a deficiency of copper and an excess of molybdenum, a combination of abnormalities that is similar to those in pastures on some peat soils and that could explain most of the symptoms in the dairy stock. On the shallower "mud" soils, which have been farmed without trouble for upwards of 50 years, no such problem has ever been notified, and the reason is probably a substantial dilution of the molybdenum during cultivation and mixing with underlying soils low in molybdenum content.

Despite the similarity of stock problems on "mud" to those on peat soils, topdressing the pasture with copper was not always successful in controlling the unthriftiness or scouring, especially in the earlier years of settlement of the deep "mud", and the possibility of some other nutritional defect had to be considered. Cobalt deficiency was one that merited examination because one of the symptoms was persistent unthriftiness, and because it has been suggested (Davis *et al.* 1956) that the cobalt requirement of cattle is increased when dietary molybdenum is high.

There was no conclusive information on the need for a cobalt supplement for stock kept on Rotomahana sandy loam either of the deep or shallow types. Many of the older farmers, located mainly on the shallow "mud", considered the soil healthy, and it is stated that bush-sick stock were sometimes sent from bush-sick pumice areas to Rotomahana mud areas to be cured. Again, on one farm that has been in operation for a number of years, sheep and milking cows have been carried without cobalt supplements and with no sign of cobalt deficiency. On the other hand, there seemed to be some association between the use of cobalt and the rearing of the best stock. The Department of Lands and Survey used cobaltised superphosphate wherever this was available and the sheep and cattle reared were good; cobalt had been used at some time on all new dairy farms during development and in the first years of settlement, and the farmers who were most successful in rearing calves were those who used cobalt at the time when it was likely to be most needed by the growing calves. It could not be determined whether this was because a cobalt deficiency existed or because by chance these farmers who were best at calf management used cobalt at the most effective time. These considerations, together with comparatively recent studies on sheep which have shown that cobalt deficiency can be borderline and cause failure to thrive without necessarily being fatal (Andrews 1956), suggested that it was desirable to make some direct tests of the need for cobalt on Rotomahana sandy loam. This paper reports the results of such tests.

EXPERIMENTAL

The work consisted of treatment trials on debilitated cattle and dosing trials in which the growth of calves or of lambs dosed with extra cobalt was compared with untreated calves on the same properties.

Therapeutic Trial

On Farm A, there were 5 heifers about 15 months old which were severely debilitated and had failed to respond to dosing with copper and had not improved when fed on very good ryegrass and white clover pasture.

Two were kept untreated as controls and three were dosed each week by mouth with a solution of cobalt sulphate containing 35 mg cobalt. Dosing commenced on 7 December 1955 and continued until 1 April 1956.

No response could be detected as a result of the cobalt dosing.

Prophylactic Trials

On each of Farms B and C, half the calves were dosed weekly from 7 December to 1 April with 35 mg cobalt per dose. There were 3 treated calves and 3 untreated calves on Farm B, and 5 treated calves and 4 untreated calves on Farm C, and they were about 5 months old at the commencement of the treatment.

No advantage to the treated calves could be detected on examinations made at least once a month, and at the end of the treatment period.

On Farm D, arrangements were made for carrying on experimental work under direction from a committee, of which the writers were members.

In 1956, 16 calves were saved, and were reared on milk and grass, weaning at about 250 lb body weight. These were allotted at birth into two groups, and 35 mg cobalt was dosed each week to the animals in one group, and the other group was untreated. All animals were weighed at approximately monthly intervals, and the body weights are given in Table 1.

It will be seen that the calves grew satisfactorily. Two in the control group died of bloat in April 1957.

There was a slight but not significant difference in weight in favour of the cobalt-treated animals, and no advantage could therefore be attributed to the feeding of cobalt.

Sheep Experiment

Because of the small, though statistically non-significant, increase in weights of the calves fed cobalt, it was considered advisable to carry out a trial on weaned lambs which provide a very sensitive indication of cobalt deficiency. Twenty-four lambs were obtained by 24 January 1957 and were divided into two groups, and 7 mg of cobalt was drenched each week to the animals in one of the groups.

Weight records are shown in Table 2.

Again there appeared to be a slight but not significant advantage to the cobalt-treated group.

TABLE 1. BODY WEIGHTS (lb) OF UNTREATED AND COBALT-DOSED CALVES

Control

Eartag No.	Date of Birth	Birth Weight	29/8/56	27/9/56	29/10/56	4/12/56	11/1/57	13/2/57	14/3/57	8/4/57	6/5/57	19/6/57	29/7/57
77190	20/7	45	82	112	155	218	270	296	332	304	364	332	360
191	24/7	55	85	116	160	225	280	280	320	Dead (bloat)	—	—	—
192	30/7	65	95	129	192	240	275	300	340	356	368	340	400
193	8/8	45	64	95	135	200	250	300	334	352	348	330	380
194	13/8	63	75	82	108	169	210	260	308	328	328	328	380
195	10/9	53	—	57	95	145	200	250	280	300	308	304	360
196	18/8	63	—	76	108	160	210	250	280	Dead (bloat)	—	—	—
197	18/9	60	-		87	140	194	230	270	288	288	280	330
Average					130	187	236	271	308	321	334	319	368

Cobalt

77180	25/7	43	77	108	150	222	290	300	332	340	348	348	380
181	28/7	54	88	125	175	250	310	340	380	400	412	420	440
182	30/7	55	80	115	155	215	260	284	312	324	308	320	300
183	7/8	63	74	110	160	232	300	320	370	380	408	390	450
184	12/8	46	57	90	125	185	240	280	320	332	328	340	390
185	12/8	63	70	105	158	220	290	330	380	396	408	380	430
186	23/8	53	—	65	102	158	210	236	260	272	268	280	300
187	26/8	65	—	73	108	167	220	260	268	280	288	285	330
Average					149	206	265	294	328	341	346	345	378

TABLE 2. Body Weights (lb) of Untreated and Cobalt-dosed Lambs

Control

Eartag No.	24/1/57	28/3/57	6/5/57	19/6/57	29/7/57
7326	55	71	76	80	83
7327	54	68	86	102	118
7328	59	77	80	83	89
7329	50	65	75	85	90
7330	50	74	80	85	91
7331	56	68	83	93	102
7332	49	62	70	83	90
7333	50	60	73	81	93
7334	53	68	78	88	100
7335	45	67	75	90	96
7336	49	60	75	86	98
7337	45	62	73	80	88
Average	51.3	66.8	77.0	86.3	94.8

Cobalt

7339	55	69	80	93	102
7340	54	72	75	86	96
7341	50	72	86	101	108
7342	58	65	85	96	107
7343	45	56	71	85	97
7344	50	62	75	85	93
7345	54	75	90	98	110
7346	50	60	75	85	83
7347	50	65	77	85	90
7348	50	68	80	86	90
7349	46	Dead	—	—	—
7350	57	80	90	105	110
Average	52.0	67.6	80.4	91.4	98.7

It was concluded that the lamb experiment also showed that no cobalt deficiency existed on the experimental farm areas.

Cobalt Content of Livers

Two livers from yearling heifers on Farm B contained 0.25 and 0.20 p.p.m. cobalt on dry matter.

One liver from 6-months calf on Farm D contained 0.16 p.p.m. cobalt on dry matter.

These figures indicated on adequate supply of cobalt.

Discussion

The dosing experiments on calves and sheep would have disclosed even a slight cobalt deficiency; these dosing experiments and the analyses of livers were consistent in showing that no cobalt deficiency existed.

The marked unthriftiness in stock which was one of the reasons for carrying out this work does not any longer occur on the area studied. Amongst the reasons suggested for this change is some maturing process associated with time and good farming practices, which tend to render the pasture more satisfactory for dairy cows and young stock. The same sequence of events appears to have occurred on a number of areas of different soil type. In each case the history is similar; on first attempting to carry dairy stock on a newly brought-in area the farmer has to contend with low production and unthriftiness, especially of young stock. The picture gradually changes with time and good management, and, after about 5 years, stock are healthy and production has risen.

An explanation of this maturing process would be valuable in the help that could be given to first settlers on other newly developed country. One of the factors in this maturation is probably a change from a clover-dominant to a more grassy sward; the present studies have shown that a cobalt deficiency is not a part of this immaturity problem.

Demonstration that there is no cobalt deficiency on Rotomahana sandy loam agrees with Grange's (1929-30) comments and with the views of older farmers mentioned in the introduction. The finding is important to fertiliser policy—many farmers use cobaltised superphosphate and incur unnecessary extra cost to themselves and extra cost to the country for cobalt importation. The experiments described show that cobalt is not necessary on Rotomahana sandy loam.

The results do not suggest that the high molybdenum in the fodder has increased the dietary requirement for cobalt.

ACKNOWLEDGEMENTS

Acknowledgement is made to Mr. B. J. Stephenson for carrying out the cobalt analysis in calf livers, and to Mr. E. D. Andrews for statistical examinations on the body-weight figures in Tables 1 and 2.

REFERENCES

Andrews, E. D. 1956: Cobalt Deficiency. *N.Z. J. Agric. 92:* 239.

Davis, G. K.; Jack, F. H.; McColl, J. T. 1956: Relation of Levels of Molybdenum to Cobalt Utilisation in Cattle. *J. Anim. Sci. 15:* 1232.

Grange, L. I. 1929: A Classification of Soils of Rotorua County. *N.Z. J. Sci. Tech. 11:* 219-28.

NITRATE REDUCTION IN THE RUMEN OF THE GRAZING SHEEP

By N. D. Jamieson, Animal Research Station, Department of Agriculture, Wallaceville.

(*Received for publication, 5 August 1958*)

Summary

Nitrate, a normal component of pasture, shows marked fluctuations in its concentration in the plant. Nitrate and comparatively small amounts of nitrite have been detected in the rumen contents of sheep on such pasture.

The effect of adding moderate amounts of nitrate to the rumen of the grazing sheep has been studied. The rumen of the sheep on pasture is capable of handling comparatively large amounts of nitrate under normal circumstances without noticeable ill-effect. Any adverse effects due to the oxidising nature of the nitrate ion were surmounted within three to four days. The results reported tend to indicate a slight stimulation of rumen function after continuous daily nitrate dosing. Under such conditions the sheep exhibited a sustained but fluctuating methaemoglobinaemia with peak values of about 35% conversion of total haemoglobin to methaemoglobin.

Introduction

Pharmacologically the nitrate ion is stated to be non-toxic, having mainly a diuretic action in the animal body (Sollman 1957). In circumstances resulting in the reduction of nitrate, toxic manifestations due to the nitrite ion formed as the primary reduction product may be obtained. Micro-organisms are capable of reducing nitrate to nitrite and this activity is shared to a small extent by tissues of several mammalian species.

In 1932, Seekles and Sjollema reported that about one-tenth of the nitrate introduced into the bovine rumen was reduced to nitrite, absorption of which converted about 20% of the blood haemoglobin to methaemoglobin. Bradley *et al.* (1939) showed the toxic principle in oat-hay poisoning to be potassium nitrate which after ingestion undergoes reduction to nitrite in the gastro-intestinal tract, with the affected animal dying from methaemoglobinaemia.

Since then the fate of nitrate in ruminal ingesta has been investigated principally by Sapiro *et al.* (1949), Clark and Quin (1951), and Lewis (1951a, b). Their work has shown that nitrite is the primary product formed during the course of nitrate reduction in the rumen, with ammonia the end-product; and that any discussion of nitrate intake in the sheep involves the consideration of nitrite metabolism. Holtenius (1957) in an extensive study of nitrite toxicity and metabolism in the sheep, was unable to demonstrate a reduced oxygen transport to the tissues of the severely methaemoglobinaemic animal and concluded that the clinical signs of nitrite poisoning are not the

result of internal asphyxiation but are caused by the vasodilatory changes induced by the nitrite ion.

Acute toxicity, generally resulting in rapid death, caused by the ingestion of plants containing very high levels of nitrate has been reported in this country (Laing and McIntosh 1947; Dodd and Coup 1957). Nitrate is a normal and highly variable constituent and metabolite of plant cells, but does not usually reach dangerous levels except in certain species. Bradley *et al.* (1939) have set the safe level for animal feeds at 1.5% potassium nitrate on the dry matter.

No references to any harmful effect on the sheep from a chronic intake of nitrate, below that required to produce acute toxicity, have been found. Provided that the degree of methaemoglobinaemia does not at any time exceed about 60% conversion of the total haemoglobin, the animal can recover (Bodansky 1951). Bradley *et al.* (1939) failed to find any evidence for a chronic toxicity due to sub-lethal amounts of potassium nitrate in cows. Abortions in recovered cows were attributed to foetal anoxia due to the methaemoglobinaemic crisis in the dam. Case (1957) mentions lowered milk production in cows, as do Muhrer *et al.* (1956) for cows on diets containing 0.75% to 1.25% potassium nitrate.

No adverse effects on the sheep due to sub-lethal doses of nitrite ion have been recorded other than a transient methaemoglobinaemia. Hueper and Landsberg (1940) have shown that chronic nitrite poisoning in rats can produce degenerative vascular and parenchymatous lesions in the heart, lung, brain, kidney, and testis, apparently due to a stagnant hypoxaemia from the slowed blood flow caused by the hypotensive vasodilation produced by the nitrite. Richardson (1941) reported that mice fed diets containing 1% to 4% sodium nitrite developed cyanosis with methaemoglobin as the only abnormal blood pigment present, and showed a moderate degree of anaemia.

Methaemoglobinaemia does not produce harmful effects other than a reduction in the oxygen-carrying capacity of the blood. Lemberg and Legge (1949) indicate that this may be more serious than is usually assumed, owing to the influence of methaemoglobin on the dissociation curve of oxyhaemoglobin. Methaemoglobinaemia does not render the erythrocytes more fragile, nor is there any direct connection between it and haemolysis (Bodansky 1951).

The present paper records some observations made on pasture-fed sheep during oral nitrate dosing with some determinations of the nitrate level in the pasture grazed and analyses of the blood and rumen contents from these animals.

METHODS

Sheep. The animals used were Romney cross sheep 1–2 years old, within the live-weight range of 70–110 lb, and were grazing a ryegrass/clover pasture which had been topdressed in early autumn with cobaltised superphosphate. Samples of rumen contents were obtained by means of a rumen tube attached to a hand pump and sample bottle. Blood samples were drawn from the jugular vein into oxalated bottles.

Potassium nitrate was given orally, by means of a rumen tube fitted with a funnel, in the form of a 5% aqueous solution. This was then followed by a similar volume of water to wash out the tube and to aid in dispersing the nitrate within the rumen. Even at the higher dose levels used, there was no toxic effect attributable to the potassium ion.

Chemical Methods. Pasture samples were prepared for the determination of nitrate, nitrite, and ammonia nitrogen by rapid freezing of a 10 g sample with dry ice. This was then pulverised and, after evaporation of surplus carbon dioxide, 5 g were weighed out and extracted with 25 ml of water for 10–15 min. This extract was then filtered and analyses were made on aliquots using the Conway (1947) methods to determine ammonia nitrogen, and after reduction with Devarda's alloy, the additional ammonia formed from nitrate and nitrite. Independent determinations of nitrite were made by a colorimetric method (A.O.A.C. 1950).

Samples of rumen contents were filtered through muslin and analyses were made on the rumen liquor so obtained. A Beckman Meter fitted with glass and platinum electrodes was used to determine pH and E_h values respectively, on the rumen-liquor sample. Total volatile fatty acids were determined by titration of a distillate of rumen liquor acidified with sulphuric acid-magnesium sulphate from the Markham still. The above methods for nitrate, nitrite, and ammonia nitrogen were also applied to 1 in 10 dilutions of rumen liquor.

The blood samples were analysed for haemoglobin by the Drabkin and Austin (1935) method, and for methaemoglobin by a modification of the method of Evelyn and Malloy (1938) using a Beckman DU Spectrophotometer.

RESULTS

The wide variation possible in the nitrate content of a ryegrass/clover pasture is shown in Table 1. The method used to determine

TABLE 1. VARIATION IN THE AMMONIA- AND NITRATE-NITROGEN LEVELS OF THE RYEGRASS-CLOVER PASTURE GRAZED BY THE EXPERIMENTAL ANIMALS

Date 1955	NH_3-N as mg N per 100 g Fresh Pasture	NO_3-N as mg N per 100 g Fresh Pasture*	KNO_3 g/100 g Dry Matter**	Date 1955–1956	NH_3-N as mg N per 100 g Fresh Pasture	NO_3-N as mg N per 100 g Fresh Pasture*	KNO_3 g/100 g Dry Matter**
13 Sept	78	30	1.1	13 Dec	138	257	9.3
15 Sept	55.5	41.5	1.5	6 Jan	115	5	0.2
21 Sept	46	40	1.4	13 Jan	113	61	2.2
26 Sept	70	29	1.1	3 Feb	81	26	0.9
7 Nov	105	100	3.6	4 Apr	109	57	2.1
14 Nov	86	25	0.9	10 Apr	82	57	2.1
29 Nov	105	27	1.0	1 May	103	23	0.8

* Determination measures nitrite + nitrate. Nitrite level negligible.

** Calculated assuming a pasture moisture content of 80%.

nitrate also includes any nitrite present. However, independent determinations of nitrite by a colorimetric method have shown that the level of this is negligible in comparison with the nitrate level of fresh pasture. Nitrite-nitrogen level was about 10–20 μg per cent compared with amounts of nitrate-nitrogen in the fresh pasture of the order of some milligrammes per cent.

It will be noted that although the ammonia-nitrogen content of the pasture varies, the variations are not as great as those shown by the nitrate, nor is there any obvious relationship between the two.

The quoted values apply only to the given sample and are not necessarily valid for the whole of any given day, or for samples from different paddocks taken at the same time on the same farm. Unquoted quantitative analyses and qualitative tests on fresh pasture have shown that there can be considerable variation in nitrate content of the plant during periods of a few hours, and marked differences between plants growing on different paddocks. It will be seen that the values found for a ryegrass/clover pasture frequently exceed the value of 1.5% potassium nitrate set as the safe limit of nitrate content of feed by Bradley *et al.* (1939).

Rumen-liquor values of nitrate-nitrogen obtained from adult sheep over the period July to April are shown in Table 2. These animals

TABLE 2. RUMEN LIQUOR NITRATE-NITROGEN LEVELS
(Samples 8.30-9.30 a.m. from sheep grazing ryegrass/clover, July 1955 to April 1956)

Distribution of Values (mg NO_3-N/100 ml Rumen Liquor)	Number of Determinations
0	19
0.1– 1.0	16
1.1– 2.0	13
2.1– 3.0	6
3.1– 4.0	3
4.1– 5.0	3
5.1–15.7	2
Range 0 –15.7	Total 62
Mean 1.4	Number of sheep 33

were sampled at about 9 a.m., at which time fermentation of the pasture eaten during the early-morning grazing period from sunrise to about 8 a.m. would be at its peak. The mean nitrate-nitrogen level was 1.4 mg/100 ml rumen liquor.

Results on rumen liquor samples from sheep grazing chou moellier fell within the range 0.4 to 4.1 mg nitrate-nitrogen per cent, with a mean value of 2.6 mg per cent.

Some comparative mean values between samples from two groups of three lambs and a pair of adult sheep are given in Table 3. These

TABLE 3. Rumen Liquor Nitrate-nitrogen Levels of Pasture-fed Lambs and Adult Sheep
(mg NO_3-N/100 ml rumen liquor)

Lamb Age (Days) / Sample Date	26 19 Sept.	76 8 Nov.	111 18 Dec.	139 10 Jan.	196 7 Mar.	215 26 Mar. 1956
Unweaned lambs (3)	0	1.3	0.2	1.0	0.1	3.0
Early weaned lambs (3)	—	2.1	0	0.4	0	3.7
Adult sheep (2)	1.0–3.3	1.7	0.4	0	0.6	0

figures show that traces of nitrate are generally present in the rumen of the sheep on pasture. The variation between individuals in a group at a given sampling was of about the same magnitude as that shown between groups. Such differences may be the result of individual selection during feeding, but are more likely to be due to individual variations in the rate of nitrate metabolism in the rumen. The sampling on 26 March indicates a more rapid metabolism of nitrate in the rumen of the adult compared with that of the lambs. Sapiro *et al.* (1949) and Holtenius (1957) have shown that the rate of disappearance of nitrate and nitrite from the rumen liquor depends largely upon the nutritional status of the rumen, being more rapid in well fed than in poorly fed sheep.

The effect of a single oral dose of potassium nitrate on some of the constituents of the blood and rumen liquor of two pasture-fed sheep is shown in Table 4. The animals selected for these experiments were apparently normal. The initial figure for total haemoglobin shows that sheep B was anaemic when dosed. The blood pigment analyses show that a marked but moderate methaemoglobinaemia was produced reaching its peak at about eight hours after dosing; the anaemic animal being affected to a greater extent owing to the smaller amount of total haemoglobin available in the blood stream. Subsequent unpublished observations indicate that the time of peak methaemoglobinaemia may vary with the individual animal, and the dose level, from four to twenty-nine hours.

The rumen liquor pH values were not altered significantly. The E_h values, given as mV relative to the calomel electrode, exhibited a marked rise presumably due to the strong oxidising action of the dosed nitrate. A return to normal values occurred within twenty-four hours. The introduced nitrate ion had largely disappeared from the rumen five to eight hours after dosing while the peak level of ruminal nitrite accumulation lay within the period two to five hours after dosing. The ammonia-nitrogen values also showed a peak over the same period. This would be consistent with the conversion of nitrite to ammonia; although such a rise is a normal feature of the post-absorptive period due to protein degradation by the rumen micro-organisms.

The following values, obtained on a post-mortem sample of rumen liquor from a sheep that died after three doses of 20 g potassium nitrate

TABLE 4. EFFECT, IN GRAZING SHEEP, OF A SINGLE INTRA-RUMINAL DOSE OF KNO_3 ON CERTAIN BLOOD AND RUMEN-LIQUOR VALUES

Sheep A.—Live weight 79.5 lb. Dosed 25 g KNO_3 at 8.30 a.m.

Time of Sampling	Blood			Rumen Liquor					
	Total Hb. g %	Met. Hb g %	Met. Hb % Total	pH	E_h mV	NO_3-N mg/100 ml	NO_2-N mg/100 ml	NH_3-N mg/100 ml	Total Volatile Fatty Acids mM/100 ml
5 min pre-dose	11.48	0.09	0.9	6.55	−225	0	0	41	10.4
5 min post-dose				6.45	−140	47	0.9	32	
2 h post-dose	11.15	0.60	5.4	7.05	− 70	22	4.5	42	
5 h post-dose	11.85	1.67	14.1	6.70	−110	7.5	8.0	48.5	
8 h post-dose	11.15	2.16	19.4	6.65	−160	1.5	0	52.5	10.3
24 h post-dose	11.15	0.19	1.7	6.50	−260	0	0	28.5	9.15

Sheep B.—Live weight 53.3 lb. Dosed 20 g KNO_3 at 8.30 a.m.
Note low figure for total haemoglobin. This apparently normal sheep was anaemic.

Time of Sampling	Blood			Rumen Liquor					
	Total Hb. g %	Met. Hb g %	Met. Hb % Total	pH	E_h mV	NO_3-N mg/100 ml	NO_2-N mg/100 ml	NH_3-N mg/100 ml	Total Volatile Fatty Acids mM/100 ml
5 min pre-dose	5.13	0.05	0.9	6.85	−250	1	0	32.5	6.7
5 min post-dose				6.70	−215	31	1.2	32	
2 h post-dost	4.85	0.42	8.7	6.65	− 55	17	9.0	39	
5 h post-dose	4.62	1.25	27.0	6.95	− 95	8.5	3.0	31	
8 h post-dose	4.69	1.58	33.7	7.18	− 5	6.5	2.5	18.5	3.0
24 h post-dose	4.69	0.05	1.0	6.80	−260	0	0	21.5	6.25

given on the previous day, do not lie outside the ranges shown in Table 4: pH 6.53; E_h 85 mV; nitrate-nitrogen 9.5 mg %; nitrite-nitrogen 7.5 mg %. The value for ammonia-nitrogen of 110 mg % was above normal, probably due to the onset of decomposition.

The values obtained from sheep A for total volatile fatty acids show no abnormality. The figures for pH, total volatile fatty acids, and ammonia-nitrogen together indicate that the anaemic animal, sheep B, did not continue to eat after dosing. It would appear that an upset in ruminal fermentation set in lasting for at least eight hours after dosing.

The results mentioned above were from animals given a single dose of potassium nitrate. In another experiment two sheep of live weights 65 and 75 lb were used as controls while two of 65 and 72 lb live weight were dosed 8 g potassium nitrate in 500 ml of water thrice daily at 8.30 a.m., 1.15 p.m., and 4.15 p.m. for three days. Rumen samples were taken during the pre-dosing period and again twenty-four hours after the last dose. Blood samples were taken at various times before, during, and after the dosing period. During dosing, the treated animals exhibited moderate degrees of methaemoglobinaemia, attaining peak values of not more than 35% conversion of total haemoglobin to methaemoglobin. Throughout the greater part of the day, however, the degree of conversion lay in the region of 9–12%. The normal and pre-dosing levels of methaemoglobin lay within the range 0 to 1%, and the raised values obtained in the dosed animals returned to these levels each day prior to the morning dose. Table 5 shows the results obtained on examination of the rumen contents. Thrice-daily dosing of potassium nitrate for three days had no effect on the pH and E_h of the rumen

TABLE 5. MEAN RESULTS FROM RUMEN LIQUOR FROM TWO SHEEP DOSED 8 g KNO_3 THRICE DAILY FOR 3 DAYS, AND TWO UNDOSED CONTROLS

	pH	E_h (mV)	NO_3-N mg/100 ml	NO_2N mg/100 ml	NH_3-N mg/100 ml	Filter Paper Digestion	Gram Stain
Controls. Pre-dose	6.35	−270	2.7	0	57	Good to excellent	Typical. Good parasitisation
Treated. Pre-dose	6.45	−260	1.5	0	41	Good to moderate	Typical. Good parasitisation.
Controls. Post-dose	6.45	−250	0	0	39	Good	Typical. Good parasitisation.
Treated. 24 hours after last dose	6.65	−240	0	0	25	Excellent	Typical. Good parasitisation.

liquor. The composition of the rumen flora as judged by the Gram stain showed no alteration, nor was the ability of the rumen organisms to digest cellulose changed. It is concluded that this treatment failed to alter the rumen flora or its function.

An experiment of longer duration was carried out in an attempt to assess the effect of continued dosing of nitrate to sheep on pasture. Two groups, each of five animals, were used. All were grazing the same

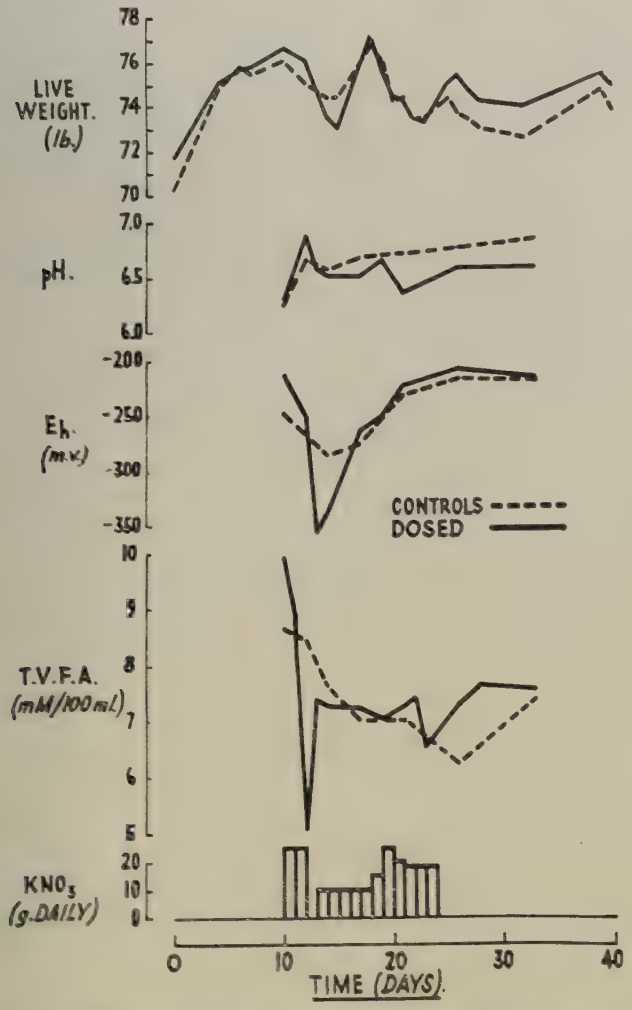


Fig. 1.—Showing changes brought about by continued nitrate dosing to sheep on pasture.

pasture during the experimental period. One of the groups served as controls while the members of the other were given daily doses of 25–10 g of potassium nitrate for fourteen days. Doses were such as to avoid the loss of experimental animals from acute nitrate poisoning. It was intended to dose 25 g daily but this proved to be too close to the lethal limit, and the dose was lowered and then raised more gradually towards this 25 g level while a careful watch was kept for any signs of impending acute toxicity in the dosed sheep.

The results are shown in Fig. 1. It will be noted that the mean live weight of the dosed group fell slightly below that of the control group on about the sixth day after treatment commenced. There were no marked changes in pH values of the rumen liquor samples. The rumen liquor E_h values of the treated animals fell to a highly negative level on the fourth day of dosing. This indicates a marked disturbance of the oxidation-reduction conditions within the rumen. Note that this is in the opposite direction to the changes found to occur within a few hours of the administration of a single dose of nitrate (Table 4). The pronounced fall in the level of the total volatile fatty acids of the rumen liquor on the third day of treatment, coupled with the relatively greater rate of decline in live weight of the dosed group on the fourth day, indicates a fall in intake on the part of the animals concerned just prior to this, i.e. during the first day or two of the nitrate regimen. However, a rapid recovery from these effects occurred and, by the seventh day of the dosing period, both groups were behaving almost identically.

The existence of this temporary ruminal upset is confirmed by the results from examinations on the Gram-stain rumen-liquor preparations from control and dosed sheep. Before treatment, all animals were showing typical Gram stains, with good to excellent parasitisation of residual plant fibres. On the second and third days of treatment, the dosed animals showed a marked drop in the degree of parasitisation of the cellulose fibres and a tendency toward an atypical Gram stain. These improved steadily from the fourth day of treatment, and thereafter all animals showed typical Gram stains with good to excellent parasitisation of the plant fibres.

Discussion

In these experiments, the difference between single or serial oral doses of nitrate solution and the comparatively smoother release of nitrate into the rumen which would occur in the animal grazing pasture equivalently high in nitrate, must be emphasised. The nitrate content of the ingested plant material would not be released into the rumen immediately, but would require time for degradation of the plant tissues during rumination and microbial digestion. Such a difference in intake may have been offset to some extent by the choice of dosing time. The nitrate solutions were given at the end of the early-morning and afternoon grazing periods, when the rumen would tend to be filled with recently grazed pasture. According to Pfander *et al.* (1956) there is no evidence of direct absorption of nitrate or nitrite from the rumen, so that, apart from a greater loss through pas-

sage of the nitrate solution further on into the digestive tract, the end-results, whether the nitrate is dispersed in solution in the rumen liquor or is being released gradually from ruptured plant cells, should be similar. Evidence has been obtained that such doses can temporarily upset the rumen flora and redox potential of the rumen liquor.

The present work shows that near-lethal doses of potassium nitrate can be handled by the rumen. In fact one of the difficulties in the above work was to avoid the sudden death of the subject by not exceeding a critical safe dosage.

During the experiments the sheep were of course exposed to the prevailing nitrate level in the pasture. Although this was not measured regularly, an indication of its possible variation has been given in Table 1. Assuming the daily pasture intake of the experimental sheep to be 800–1200 g dry matter, a dose of 20–25 g of potassium nitrate would be equivalent to an additional pasture content of 2–3% potassium nitrate in the dry matter.

Sustained dosing at this level produces a transient fall in the quality of the rumen flora and in the efficiency of the rumen fermentation. It would appear that the rumen organisms rapidly become adapted to handling these amounts of nitrate, and once this has occurred the animal on pasture shows no adverse effects from the increased nitrate metabolism nor from the concomitant methaemoglobinaemia. These findings for the pasture-fed sheep are supported by those of Clark and Quin (1951) who reported a slow gain in weight in sheep on a diet of hay, molasses, and 4% sodium nitrate.

ACKNOWLEDGEMENT

The author is indebted to Mr. T. M. Loftus for valuable technical assistance.

REFERENCES

Association of Official Agricultural Chemists 1950: "Official Methods of Analysis", 7th ed.

Bodansky, C. 1951: Methaemoglobinaemia and Methaemoglobin-Producing Compounds. *Pharmacol. Rev. 3:* 144-96.

Bradley, W. B.; Eppson, H. F.; Beath, O. A. 1939: *J. Amer. vet. med. Ass. 94:* 541-2. (*Wyo. agric. Exp. Sta. Bull. 241.*)

Case, A. A. 1957: *J. Amer. vet. med. Ass. 130:* 323-9.

Clark, R.; Quin, J. I. 1951: *Onderstepoort J. vet. Sci. 25:* 93-103.

Conway, E. J. 1947: "Microdiffusion Analysis and Volumetric Error". Rev. ed. p. 155. Crosby Lockwood, London.

Dodd, D. C.; Coup, M. R. 1957: *N.Z. vet. J. 5:* 51-4.

Drabkin, D. L.; Austin, J. H. 1935: *J. biol. Chem. 112:* 51-65.

Evelyn, K. A.; Malloy, H. T. 1938: *J. biol. Chem. 126:* 655-62.

Holtenius, P. 1957: *Acta agric. Scand. 7:* 113-63.

Hueper, W. C.; Landsberg, J. W. 1940: *Arch. Path.* (*Lab. Med.*) *29:* 633-48.

Laing, A. D. M.; McIntosh, I. G. 1947: *N.Z. J. Agric. 74:* 377-8.

————1957: *N.Z. Dep. agric. annu. Rep. 1956–7,* p. 42.

Lemberg, R.; Legge, J. W. 1949: "Hematin Compounds and Bile Pigments". pp. 519-20. Interscience Publishers, London.

Lewis, D. 1951a: *Biochem. J. 48:* 175-80.

————1951b: *Ibid. 49:* 149-53.

Muhrer, M. E.; Garner, G. B.; Pfander, W. H.; O'Dell, B. L. 1956: *J. Anim. Sci. 15:* 1291-2.

Pfander, W. H.; Ellis, W. C.; Garner, G. B.; Muhrer, M. E. 1956: *J. Anim. Sci. 15:* 1292.

Richardson, A. P. 1941: *J. Pharmacol. 71:* 203-9.

Sapiro, M. L.; Hoflund, S.; Clark, R.; Quin, J. I. 1949: *Onderst. J. 22:* 357-72.

Seekles, L.; Sjollema, B. 1932: *Arch. wiss. Prakt. Tierheilk. 65:* 331-43. Seen only in *Nutr. Abstr. Rev. 3:* 329.

Sollman, T. 1957: "A Manual of Pharmacology". 8th ed., pp. 1168-9. W. B. Saunders, Philadelphia.

INTRASPECIFIC COMPETITION AS A FACTOR IN RYEGRASS BREEDING

By S. O. Fejer, Grasslands Division, Department of Scientific and Industrial Research, Palmerston North.

(*Received for publication, 16 June 1958*)

Summary

In a study of intraspecific competition, using perennial and hybrid ryegrasses, three planting techniques were tried in three field experiments. The first experiment measured totals of groups of competing plants and the technique can be used for progeny testing in place of plot tests when seed supply or space is limited, and by varying the number of plants within groups, changes in competition intensity can be studied. The second and third experiments were intended for aiding selection work. Measurements were made on individual central plants in similar small groups and in larger groups, including some plants exposed to interspecific competition by alternate clover rows. It was concluded that single plant tests do not give precise information on the behaviour of the same plants under competitive conditions of varying severity. The deviations can amount to highly significant interactions and their degree depends on the plant material used. The main factor in ryegrasses seemed to be tillering habits and F1 hybrids showing heterosis effects were particularly subject to interactions of such characters with spacing. A new method for measuring tiller number was also evaluated here.

Certain shortcomings of the planting techniques were pointed out, especially in the border effects, and possible improvements were discussed. Potentialities of an "evolutionary plant breeding method" suggested by Suneson (1956) and possible changes during seed increase in strains were considered, while presenting some data on the influence of competition on reproductive growth.

Introduction

Intraspecific competition is rarely considered by the plant breeder but it is in fact important in selection and also in seed multiplication. In self-fertilised plants the relative uniformity within varieties makes the study of competition easier than in cross-fertilised plants. Even so, only a few of the factors have been studied. With cross-pollinated pasture plants, the genetic variability of the material, the density of the swards, and the possible influence of interspecific competition, make the problem very complex.

With ryegrass in New Zealand, Brougham (1954) in an experiment concerned with the effect of seeding rate on pasture establishment, studied some aspects of intraspecific competition. In addition, a technique for evaluating one specific aspect, base-shading, was presented by Mitchell and Coles (1955), who reported an increase in variability of growth in spaced plants. The plant-breeding procedure used in New Zealand for cross-pollinating pasture plants has been described by Corkill (1957). Evaluation of plants and progenies is based mainly on the appearance, disease resistance, and productivity of spaced plants, although progenies are also studied as swards when

sufficient seed is available. Sears (1951) observed that although species comparisons based on spaced plants could be invalid, the results of this New Zealand plant-breeding procedure have been remarkably good.

The main object of the present work was to determine the importance of intraspecific competition in ryegrass performance.

Experimental Procedure and Results

Trial 1 (1952-3)

This trial was designed mainly for testing general competition effects. Miniature plots were planted into cultivated ground. The competitive effects were achieved by increasing numbers of plants per plot, the spacing within the plots being constant.

Methods

Diallel-cross progenies of four perennial ryegrass plants A, B, C, and D, collected from old pastures, were grown as spaced plants, 2 ft apart. Detailed results from these have been reported earlier (Fejer 1958). Two of these progenies, B × D and C × D, and three further crosses to plant E, from the same collection, A × E, B × E and C × E, were planted in July 1952 in three groups, 2 ft 6 in. apart. The groups used were 2 × 2, 3 × 3, and 4 × 4, i.e. 4, 9, and 16 plants as a unit, the spacing between plants being $1\frac{1}{2}$ in. within each unit. This spacing in a sward is equivalent to surviving plants from a seeding rate of 15 lb per acre, and thus will ensure moderate pasture competition. Two randomised blocks of the 15 cross × group combinations, situated next to the spaced plants trial, provided 10 replications of the groups. Measurements, taken at intervals of 3 to 6 weeks until February 1953 were: green weight of the herbage cut to 1 in. in height, tiller number, tiller weight, spreading, density, and number of emerged heads. The individual plants within the groups were not cut separately, but mean values were calculated for them.

Results

These are presented graphically in Fig. 1 on a log. scale on a per plant basis for September and November for green weight and tiller number, and on a per unit basis for December for head number and percentage of heading tillers.

Discussion

The curves for green weight show that with increasing competition the production of the individual plants in each unit is reduced in proportion to the number of plants in the unit. Tiller number per plot behaves similarly to green weight per plant. Brougham (1954) also measured tiller number at this station in ryegrass pastures in the same months, and presented graphically the influence of various seeding rates on tiller number. His data are very similar to those of tiller numbers in the present Fig. 1 if the log transformation is allowed for. It is realised that the two ways of modifying competition intensity are of entirely different nature—varying border effects against varying

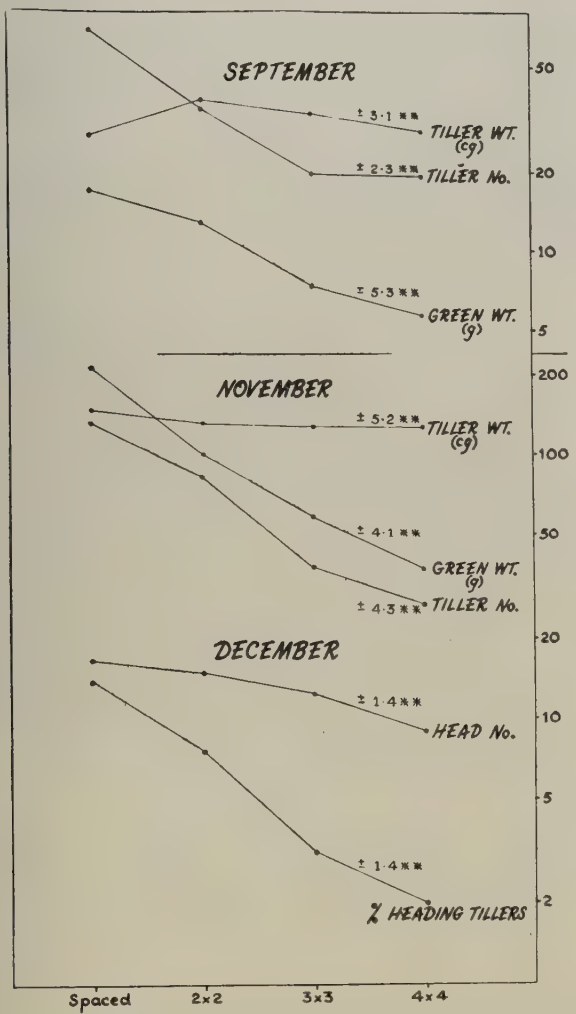


Fig. 1.—Green weight, tiller weight and number per plant (Sept., Nov.), and head number and percentage of heading tillers (Dec.).

spacing—but it is thought that the competitive forces involved (light, water, nutrients, etc.) would be essentially similar. Even if the competition intensity is higher in pastures, the similarity of its effect justifies the assumption made.

Tiller weight is obviously less directly influenced than tiller number but the effect of the different seeding rates applied by Brougham becomes more marked after the early stage than that displayed in Fig. 1 between spaced plants and groups.

In general, the differences between the means of the plants in groups 3 × 3 and 4 × 4 are less than those between lower intensities, so that, for practical purposes, the 3 × 3 group planting shows some promise for progeny testing, any greater number of plants per group not giving much added efficiency.

As well as the comparisons of seeding rate effects there is another theoretical way of analysing the nature of the competition operating in the groups. This is based on calculations on the relationship of each plant to its neighbours or to bare ground. If we allot arbitrary competition values:—

0 = single plants (no competition, surrounded by bare ground)

3 = corner plants (competing with 3 surrounding plants)

5 = border plants (competing with 5 surrounding plants)

8 = central plants (competing with 8 surrounding plants)

then, considering the number of plants with different values in each group, mean competition intensities could be calculated for these groups:

Spaced plants = 0

2 × 2 group = 3

3 × 3 group = 4.4

4 × 4 group = 5.25

In September there was a highly significant regression of plant weight on these competition intensities ($F = 122.0$, d.f. 1, 27). Taking only the nearest plants into account (0, 2, 3, 4 instead of 0, 3, 5, 8 as above) made little difference in the conclusions.

Trial 2 (1953-4)

In this trial a wider range of material was used and comparisons were made between spaced plants, 3 × 3 groups, and single plants under competition (i.e. the centre plants of the groups). Attention was also paid to possible heterosis effects in the two F1 progenies.

Methods

One highly productive ryegrass plant of perennial (*Lolium perenne* L.), short-rotation (*L. perenne x multiflorum*) and Italian (*L. multiflorum* Lam.) origin was open-pollinated in its respective increase area (see Corkill 1950), giving progenies "1", "3", and "5". These same plants were also intercrossed, "1" × "3" giving progeny "2", and "3" × "5" giving progeny "4". Seedlings of these progenies were planted out in June 1953, as spaced plants and as square groups of 9 plants (see "3 × 3" in Trial 1), in a "2 × 5" split-plot design with 20 blocks and two units of a progeny per plot. Planting distances and cutting measurement procedures were similar to those in Trial 1, with additional dry-matter determinations, plant-height measurements, and tiller-thickness (= tiller weight/plant height) calculations. The centre

TABLE 1. MEANS OF HERBAGE YIELD AND ITS COMPONENTS IN SPACED PLANTS AND UNDER COMPETITION

Date	Spaced Plants						Centre Plants of Groups					
	Strain						Strain					
	1	2	3	4	5	S.E.	1	2	3	4	5	S.E.
August 1953						±						±
Green weight (g)	10.5	16.9	16.5	19.7	24.0	0.9**	5.8	9.6	10.5	12.0	10.5	0.7**
Tiller number	44	55	60	50	42	3.1**	29	40	39	35	25	2.8**
Tiller weight (mg/cm)	20	19	18	23	32	1.1**	14	14	14	18	23	0.7**
Plant height cm	12	15	15	17	18	0.4**	14	17	19	19	19	0.6**
October 1953												
Dry weight (g)	32	53	43	52	53	2.5**	5.8	7.3	8.5	8.0	7.0	0.4*
Tiller number	293	410	238	218	273	14.1**	82	73	56	43	29	5.8**
Dry tiller weight (mg/cm)	3.6	3.5	4.9	6.4	7.7	0.2**	2.5	2.4	3.6	4.1	4.8	0.2**
Plant height cm	32	37	37	38	41	0.8**	35	41	40	44	49	1.0**
December 1953 (heading)												
Dry weight (g)	102	165	149	156	171	8.8**	12.2	14.5	16.3	16.4	10.5	0.7*
Tiller number	428	735	461	363	324	30 **	76	67	63	53	26	5.6**
Dry tiller weight (mg/cm)	3.7	3.1	4.4	6.0	6.3	0.2**	2.6	3.2	4.2	5.1	6.2	0.4**
Plant height cm	65	77	73	72	84	1.5**	63	70	62	60	65	2.9
Percentage of heading tillers	62	63	59	66	64	3.0	28	44	37	35	40	3.1**
April 1954												
Green weight (g)	32	96	43	44	16	6.8**	2.9	4.5	3.2	2.6	0.5	0.8*
February 1955							Total of 9 plants in groups					
Green weight (g)	32	120	81	162	127	29.6	64	121	75	137	150	7.8**

** Significant at the 1% level.
* Significant at the 5% level.
(*) Significant at the 10% level.

These signs will be used throughout this paper.

plant of each group was marked with a wire ring, cut with scissors and measured separately.

Results

Table 1 gives the herbage yield and its components until April 1954, of the spaced plants and the centre plants of each group. The values of the group totals, most of which were similar to the spaced-plant values, are not given here except for the final cut in February 1955 when centre plants were not cut separately. However, they were considered in a combined Analysis of Variance of the October and December dry weights shown in Table 2. The data were

TABLE 2. ANALYSIS OF VARIANCE OF OCTOBER AND DECEMBER DRY WEIGHTS (transformed to "log. dry weights")

Source of Variation	d.f.	Mean Square	Result
Blocks	19	0.132	**
Plants	4	0.466	**
Competition	2	101.707	**
Plant × Competition	8	0.131	*
Error (1)	266	0.063	
Dates	1	18.613	**
Date × Plant	4	0.028	
Date × Competition	2	0.889	**
Date × Plant × Competition	8	0.035	*
Error (2)	285	0.014	

transformed to logarithms because of the difference in production between the two months. Also from the weight results of Table 1, a "Competition index" ("C.I.") was calculated being the yields of the centre plants as percentages of the group totals. This should give an indication of changes in competition intensity.

TABLE 3. COMPETITION INDICES (C.I.) CALCULATED FROM TABLE 1; CENTRE PLANTS AS PERCENTAGE OF GROUP TOTALS

Strain	1	2	3	4	5	S.E. ±	Coefficient of Variation
August 1953	13.9	14.7	17.0	14.1	9.8	2.2**	36%
Oct. 1953	6.7	6.5	8.5	7.4	6.5	1.6	51%
Dec. 1953	9.5	7.9	8.7	8.3	5.6	2.2	63%
April 1954	6.3	4.4	5.3	8.3	1.6	3.1*	133%

Finally, measurements from the reproductive stages of growth are given in Table 4, for the spaced plants and totals of groups only.

TABLE 4. REPRODUCTIVE CHARACTERS IN SPACED PLANTS AND GROUPS
(Means and standard errors with indication of significance)

Date	Spaced Plants						Totals of 9 Plants in Groups					
	Strain						Strain					
	1	2	3	4	5	S.E.	1	2	3	4	5	S.E.
November 1953						±						±
Date of emergence	7	9	20	24	24	3.0**	9	13	19	26	25	3.0**
January 1954 (heading)												
Plant height cm	50	60	60	63	73	1.8**	46	57	64	64	64	1.2**
March 1954 (heading)												
Plant height cm	30	42	44	48	48	1.7**	35	41	48	48	50	1.2**
February 1955 (heading)												
Head number	10	208	142	207	213	22.8**	53	160	157	247	214	22.8**
Seeds per head	4.6	14.1	14.0	22.0	17.3	2.0**	15.9	9.4	16.5	17.8	21.5	2.0*
Wt. of 100 seeds mg	170	174	181	229	256	7.8**	192	209	190	230	259	7.8**

Discussion

The most interesting information is summarised in Table 2 showing that there were significant plant × competition and date × plant × competition interactions. From the true mean weights in Table 1, it is apparent that these interactions were mainly caused by the "1" × "3" hybrid—Strain 2. Under spaced-plant conditions this hybrid outweighed both parents in October and December while no such heterosis effect was observed in the centre plants. For green weights in August, there was no heterosis effect in either planting while in April there was a pronounced heterosis in spaced plants. In February 1955, there was (green weights) an indication of heterosis for spaced plants compared with a definite heterosis for the total of 9 plants in group. Tiller measurements were made only until December and indicate that the hybrid vigour up to that time was mainly due to the superior tillering ability of the hybrid. Restriction of tillering by competition was not compensated for by tiller weight.

The opposite heterosis situation can be found in August for green weight in the "3" × "5" hybrid (Strain 4), a moderately tillering, high tiller-weight progeny. For spaced plants Strain 4 was significantly different from Strains 3 and 5 and its value was intermediate, whereas for centre plants there was a strong indication of heterosis. No significant differences exist between the two plantings for the months October, December, and April for heterosis or intermediacy but in February 1955 the spaced planting showed an indication of heterosis.

For December it was calculated that on the "1" × "3" side there was a significant dominance in spaced plants but not in the centres, while on the "3" × "5" side there was a highly significant dominance in centres but not in spaced plants, and consequently there was a highly significant bias in the dominance × competition interaction. The yield components point again to the tiller number as responsible, but the superiority of the hybrid this time was expressed by the ability to withstand competition with less reduction in tiller number than in the Italian parent.

One could generalise that heterosis, or at least dominance in the hybrids may be expressed either only in spaced plants or only in pastures, depending on whether the hybrid progeny's main feature of adaptation is tiller number or tiller weight. While more information will be needed, it should be considered in the present case whether the features of the group planting system applied have anything to do with the results. In contrast to the groups in Trial 1, only the centre plant of each group was measured. With the wide range of growth types and the fixed distance between plants (1½ in.) it is possible that in the groups of the types requiring more space, like Italian, the centre plants are penalised through the vigorously growing border rows. Even within a strain, the border row could vary in this respect from group to group, but this would only increase variability in the centres and contribute to the experimental error. Similar, too, would be the effect of environmental influences such as soil variability on the groups. For example it was observed that in strains 2 and 5 there was a particularly high variability between patches of apparently different soil fertility.

Calculation of the correlation between yields of spaced plants and centre plants of these progenies from block to block in December resulted in a nearly significant negative coefficient ($r = -0.22$, d.f. 37), verifying the supposition that where the environment favoured the spaced plant, the centre plant has been penalised by the more vigorous border.

While such errors can influence the precision of selection, requiring higher numbers or replications, the real danger falsifying the correct conclusion, as stated before, would be if a whole strain would be penalised by the group planting. The C.I. values (Table 3) give some information on this matter. The Italian ryegrass has clearly, for August and April, been depressed in growth compared with the other strains, while the slight reduction in October and December could hardly influence the previous conclusions on the interactions between strains in these months. Still, the declining percentage of all the centre plants, as shown in Table 3 and the parallel increase in the coefficients of variation indicate caution in interpretation of results.

Regarding the reproductive characters, the general influence of competition is rather straightforward in the reduction of the percentage of heading tillers in the centre plants in December (Table 1), while in characters where only the group as a whole could be measured, like the date of emergence and plant height in aftermath (Table 4), this retarding influence is slight, or absent, especially in the later aftermath when there is an overall reduction of plant heights. One should not forget that in these characters because of the sampling method the groups are represented by the earliest and tallest plants which are most probably in the less retarded but more variable borders.

The seed data in February include all component factors, so that their multiplication results in total seed production. At this time of aftermath heading perennial ryegrass is largely vegetative. This might also have influenced some of the component characters which showed highly significant differences between the strains. However the combined analysis revealed some significant interactions which may be relevant. The change in the heterosis effect of hybrid 2 in number of seeds per head—nil in spaced plant but negative in the groups—is somewhat similar to that discussed in green weight. While the effect in green weight was actually determined by tiller number the effect on seed per head might be influenced by head number to which it usually is negatively correlated. Head number is the character which is primarily reduced in the aftermath of perennial ryegrass. Apparently in the present case this reduction was stronger in the spaced plants than in the groups, contrary to expectation, and in contrast to the other progenies. This could perhaps be due to the postponement of the short heading period by competition, but whatever the cause, it could interfere with the above interaction.

Trial 3 (1954–5)

In this trial an attempt was made to reduce the uneven and very strong border effect, experienced in Trial 2, by planting 64 plants in a group instead of 9. The "1" × "3" material, showing the highest

interactions in Trial 2, was used, but from a wider crossing programme and from the F2 generation, to exclude the heterosis effect in the F1. This time the size of the groups also allowed the inclusion of alternate clover rows, to approximate actual pasture conditions in this respect. To do this, the technique of Caputa (1948) was adopted and extended to allow for various ryegrass/clover ratios. This was done not so much for studying the different degrees of interspecific competition or co-operation, but it was hoped that the optimum ratio allowing separate measurements on individual ryegrass plants would be found. The layout also gave more scope for the study of changes in variability under competition than in Trial 2.

Methods

Seed of New Zealand pedigree perennial ryegrass and seed harvested in a polycross of selected F1 plants in a "1" × "3" crossing programme, was sown in boxes and planted out in the field in October 1954, as spaced single plants 2 ft apart, and as groups. The groups each contained four rows of 8 ryegrass plants together with varying numbers of clover plants in intervening rows to give grass:clover ratios of 1:1, 2:1, and approx. 3:1. The rows of plants within the blocks were 1½ in. apart. In the alternate rows with only 3 or 4 clover plants, the distance between these was accordingly 4½ or 3 in. White clover was used in all 3 ratios, while red clover was tried in the 2:1 ratio only, giving 4 different types of groups. The distance between the groups in the field was 4 ft and the layout was in three replications of 2 × 2 split-plot design, the main plots representing the two strains and the sub-plots containing 32 spaced plants of 4 different group types.

Green weight, tiller number, and tiller weight determinations were made from November 1954 till April 1955. When possible within the groups, clover and ryegrass were weighed separately and 18 (3 × 6) centrally situated ryegrass plants were measured individually, but this was not always feasible and by April most of these central plants died following a very dry summer. The measurement technique was the same as in Trials 1 and 2, but at the April cut of the spaced plants the procedure for the tillering data was reversed. Dry tiller weights were taken of two random 10-tiller samples from each plant, to the nearest 5 mg, and tiller numbers were calculated, on a constant 20% dry matter basis for all plants. This accelerated the work substantially. A component of variance analysis showed that taking two samples per plot was less important than the number of plants measured, as shown in the following variances of the means for various numbers of plants per plot (p) and samples per plant (s):

p	1	4	10
2	0.00220	0.00198	0.00193
8	0.00055	0.00049	0.00048
50	0.00008	0.00007	0.00007

Results

There were no differences in the ease of finding individual ryegrass plants among the groups with different clover ratios. An analysis

TABLE 5. MEAN GREEN WEIGHT (g) AND ITS COMPONENTS (TILLER NO. × TILLER WEIGHT) IN DIFFERENT MONTHS IN TWO PLANTING METHODS

Date	Single Plants		Groups	
	1	1 × 3	1	1 × 3
Nov. 1954	7.4 (68 × 0.092)	8.4 (68 × 0.119)	7.0 (32 × 0.206)	8.3 (31 × 0.244)
Dec. 1954	36.2 **	48.7	6.1 *	8.9
Jan. 1955	45.1 *	65.8	11.9 *	16.2
Mar. 1955	32.7 *	50.3	2.0 ** (24 × 0.063) (*)	4.9 (28 × 0.118)
Apr. 1955	43.0 ** (266 × 0.175) **	65.5 (276 × 0.237)	8.1	10.4

Significant differences between strains within treatments indicated by asterisks under characters in question.

of variance has been made to include the possible effect of the different clover ratios but the results do not affect the comparison of single plants and groups and are thus not presented. The main ryegrass results are summarised in Table 5.

Discussion

The analysis of the single plants and groups was done separately throughout, but a comparison of the two sets of data shows that no major interaction occurred between the planting methods, comparable to Trial 2. In each season the same characters were significantly higher, or equal, in the hybrid compared with Strain 1 in both plantings. This can be interpreted as the result of using F2 material and the consequent lack of heterosis in tiller number under any planting. However, the lack of planting interactions in the "mean yields" does not mean that interactions influencing the within-strain selection of individual plants were not present. On the contrary, they are indicated by the entirely different growth form of the two plantings in different seasons. The initial effect of competition in this trial—as in Trial 2—was an increased tiller weight as compared with the spaced plants. That this was caused by the high growth rate of leaves on the centrally situated plants--presumably an effect of mutual shading and etiolation --was already shown in detail in Table 1 of Trial 2 under plant height in August and October but not in December when the heading plants were higher in the spaced plants than in the centre plants of each plot. In the present trial only the average plant height was recorded, and only at the first two cuts when the differences were still pronounced. These were 5 in. for singles and 12 in. for groups in December when these spring-planted plants remained vegetative. By autumn the situation reversed and tiller weight was much higher in spaced planting.

It was hoped that some indication of different selection possibilities would result from comparisons of variabilities of the tillering characters. The coefficents of variation were higher under competition, and the difference increased with the season. There was a tendency in the groups, but not in the single plants, for the tiller number measurements to be more variable than the tiller weight measurements. This tendency was especially marked in the hybrid, and in Table 6 a t-test shows a significant interaction of these coefficients of variation. That

TABLE 6. Coefficients of Variation of Tillering Characters of Strain 1 × Strain 3 Under Different Conditions

	Single Plants		Groups	
	Tiller No.	Tiller Wt	Tiller No.	Tiller Wt
November 1954	36% ± 1.9	40% ± 2.0 (d.f. 189)	55% ± 2.8	49% ± 2.5 (d.f. 210)
t. test of interaction	2.17*			
March 1955 (Groups)	48% ± 3.5	48% ± 3.5 (d.f. 93)	132% ± 6.4	93% ± 4.5 (d.f. 210)
April 1955 (Spaced)				
t. test of interaction	8.13**			

the spatial restriction of tiller number should increase its relative variability appears curious at first thought and it seems interesting in this regard to point out that Mitchell and Coles (1955) stress the fact that in Strain 3 under artificial base shading—which does not involve root competition greater relative variation was found in tiller weight than in tiller number. Again in Trial 2, Table 1, tiller number was much more variable than tiller weight both in spaced plants and in centres, in all strains. Thus it seems that both degree and kind of competition and the plant material used can influence this relationship between the variabilities of tillering characters, so that no valid conclusions as to methods of selection can be drawn.

Referring to the clovers, it should be noted that under the management adopted, white clover faded out as the season advanced while red clover reached its peak in the autumn in spite of competition. There was also a tendency for a decrease in ryegrass growth with increase of the clover ratio, similar to the effect of varying ryegrass seeding rate on clover growth, reported by Brougham (1954). These interspecific competitive effects significantly decreased the green weight of the ryegrasses in the plots with the highest white-clover ratio in November. Similarly the tiller weight in March was depressed in the red clover plots.

General Discussion

Competition can hinder breeding success in three main ways:

(1) The behaviour of selected parent plants and their progenies may be different under competitive conditions from that under the wide spacings used during breeding work.

(2) Even if there are no major ranking differences in the basic material under competition, it is possible that parent plants selected under competition would produce a strain more suitable for competitive conditions. A technique that could be used in a large-scale plant breeding programme has still to be devised.

(3) Competition between genotypes may induce significant changes during seed multiplication. This is a problem but it can be also regarded as a crude technique for (2).

In pasture plants, interest was formerly concentrated on competition among the many species of the complicated seed mixtures. Only with the advent of modern simple mixtures has the competition within one species been considered. Most published work falls under point (1) above, dealing with possible differences between spaced and competitive plantings in comparisons of different strains or progenies. This is also a problem in self-fertilising cereals, e.g. Whitehouse (1953) quoted instances where in barley the order of yields was reversed when changing the method of sowing. He also stated that drilling resulted in a better separation of strains than spacing. There seem to be differences of opinion among those who have worked with cross-pollinating plants. McDonald *et al.* (1952), Grissom and Kalton (1956) and Frandsen (1956), among others, think that for measuring yield and other polygenic characters, spaced plant tests are of little use, while Murphy (1952) and Lazenby (1957) found no difference between spaced and broadcast tests. Lazenby, discussing these different views, thinks that many of the different results found by American workers come from errors in eye estimation, while differences in material and environment might also be important.

Information from the present trials on this point show that the material used is of overwhelming importance for the presence or absence of interactions in ranking due to competition. The decisive factor here is the tillering habit of the individual and the way this is influenced by the other partners in the community. Even Lazenby finds a relatively higher production of short-rotation ryegrass when compared with S24 ryegrass in plots, than in single plants, and explains this by the high variability of the short-rotation ryegrass, which would reduce the competition for limiting factors like water. In the present Trial 2, as discussed, there were strong indications that strains with high tiller number suffered relatively more from competition than those with high tiller weight under the above conditions. This relationship would then explain Lazenby's results as there is a marked difference in tillering between S24 and short-rotation ryegrass.

In the present work, spacing interactions were especially marked between first-generation hybrids and their parents, possibly due to heterosis effects, while older strains or the second generation in a hybrid line showed more consistency in yield differences between

plantings. It is thought that newly arisen, unselected, genetic variation is generally more likely to show similar interactions, and it might not be a coincidence that some of the most striking ones reported were found in artificial mutations produced by irradiation by Gustafsson (1954). If this is so, in breeding programmes utilising similar variation through hybridisation, induced mutations, or polyploidy, testing under competition may be of importance. As seed supply in such material is usually limited, progeny-testing techniques like those reported in Trial 1 may be useful.

Gustafsson (1953, 1954) also pointed out the necessity of considering competition effects in biometrical calculations. This leads to the second point of how to test individuals under competition. The problem has already been recognised by Keller (1946) and Donald (1951) and it has been developed in a number of experiments in cereals by Sakai and co-workers (1953, 1954, 1955). The aims of Sakai were in many ways similar to the present ones. In his 1955 paper he varied the number of competitors around a plant in a hexagonal pattern—obtaining different competition intensities in a confined space similar to the technique in the present Trial 1 and he was able to separate a "competitional variance" in the biometric analysis. However, as was pointed out in the discussion of that paper by Mather (1955), interpretations from the pure lines used there are much less conditional than those dealing with the complexities of segregating families.

In the present work, the variation in competition intensity in Trial 1 may have applications where the behaviour of progenies is compared in that respect, but in the attempts in Trials 2 and 3 to select individual plants under competition, the difficulties due to the heterozygous material used—pointed out by Mather above—became apparent. As discussed after Trial 2, even if the general argument as to the necessity of selecting under competitive conditions was strengthened in these trials, there were various factors contributing to the steadily increasing random variation, magnifying the error term, and shown in the coefficients of variation in Tables 3 and 6. Only one of these factors, the effect of unrestricted border, was lessened by the larger groups of Trial 3 and while this factor could be eliminated if the trials were laid down amidst continuous swards, the advantages would hardly outweigh the increased technical difficulties.

The high error term necessitates the growing of a large number of plants for selection under competition, preferably also in clonal replications. Whether the number required is forbiddingly high, cannot be said without conducting suitable breeding experiments. A selection for high productivity has been started from all the plants of 1×3 in Trial 2, and in clonal glasshouse tests under different environments significant differences were found in tillering and light response between the best plants from spaced plantings and from centres (Fejer, unpublished). But without further crossing and testing it is difficult to say how far such differences may be after-effects of the previous growing conditions and how far they represent heritable competitive advantages.

In any case, improvements of the testing technique presented here seem to be essential in heterozygous material, to reduce work and increase efficiency. It could be suggested that a relatively uniform "tester line"—preferably one with a distinct leaf character—be used to reduce the random genetic differences in the border, but this is a difficult proposition if the introduction of a new "tester × tested lines" interaction term is to be avoided. Actually, with N.Z. ryegrass material, the use of the Irish "Golden Base" strain as border has been attempted (Fejer, unpublished) but the large difference in vigour between tester and tested lines made the competition effect unreliable. A much more radical change in testing technique would be the artificial application of controlled competitive conditions as attempted for one factor in the work quoted earlier, by Mitchell and Coles (1955). This does away with the random environmental effects altogether, but much more work of the kind done by Hodgson and Blackman (1956, 1957) in *Vicia faba* would be needed to elucidate all the main factors of competition and even then the network of controlled conditions required may be too complex for practical applications.

There is a practical solution suggested by Suneson (1956) in his "evolutionary plant breeding method" which represents a transition to the third problem set out at the beginning of this discussion, that of the mutual competition between genotypes. The effect of such competition under natural conditions in the formation of different ecotypes has been accepted by many workers since the early work of Turesson (1922), and it has been used by the plant breeder in collecting his initial material in places where the conditions are similar to those prevalent in the area where he intends to use his product, such as in old pastures for a "pasture" strain of ryegrass. If the forces of natural selection could be harnessed and applied at will, this would mean saving work and increasing efficiency at the same time. The production of convincing evidence on this possibility must necessarily be a long-term project in cross-fertilised plants, but certain special problems can be easily solved by such methods as shown by Burton's work (1948). He assessed the suppression of inbred material by outcrossed material in maintaining hybrid vigour in F1 bulks of *Pennisetum*.

Much more work of this kind is known in autogamous cereals where it is often necessary to decide whether pedigree or bulk breeding methods are more efficient, e.g. Frankel (1935), Suneson (1949), Sakai (1951), Palmer (1952) and Van der Kley (1955). The outcome of such free competition through a number of generations must be the combined effect of competitive ability at vegetative stages and of its effect on reproductive ability as shown by Gotch (1956) in the case of degeneration of a barley variety due to undesirable genotypes of high competitive and reproductive ability.

This is a problem of its own in the strain building procedure used in ryegrass where several generations of seed multiplication are necessary to bring the product on the market. No direct information is available from this work, but the results discussed above on differences in competitive ability in vegetative characters, combined with the effect of competition on reproductive characters, discussed after

Trials 1 and 2, make it most probable that some changes usually occur during this procedure. More knowledge on the effect of competition on reproductive ability as reported by Donald (1954)—would make such changes more predictable.

Acknowledgements

Thanks are due to Mr. A. C. Glenday of the Applied Mathematics Laboratory of the Department of Scientific and Industrial Research for aid in calculation and planning and interpretation of various aspects of these trials. The assistance in field measurement of Mr. J. M. Todd and Mr. C. S. Armstrong of Grasslands Division is gratefully acknowledged.

References

Brougham, R. W. 1954: Pasture Establishment Studies. I. The Effect of Grass Seeding Rate on the Establishment and Development of Pastures of Short-Rotation Ryegrass, Red and White Clover. *N.Z. J. Sci. Tech. A35:* 518-38.

Burton, G. W. 1948: The Performance of Various Mixtures of Hybrid and Parent Inbred Pearl Millet, *Pennisetum glaucum* (L) R. Br. *J. Amer. Soc. Agron:* 903-15.

Caputa, J. 1948: Untersuchungen uber die Entwicklung einiger Gruser und Kleearten in Reinsaat und Mischung. *Landw. Jahrb. Schw:* 849-976.

Corkill, L. 1950: A Comparison of Methods of Progeny Testing for Quantitative Characters in Ryegrass (*Lolium* spp.) *N.Z. J. Sci. Tech. A32 (3):* 36-44.

———1957: Pasture Plant Breeding in New Zealand. *Agric. Rev. 3:* 31-37.

Donald, C. M. 1951: Competition Among Pasture Plants. I. Intra-specific Competition Among Annual Pasture Plants. *Aust. J. agric. Res. 2:* 355-76.

———1954: Competition Among Pasture Plants. II. The Influence of Density on Flowering and Seed Production in Annual Pasture Plants. *Ibid. 5:* 585-97.

Fejer, S. O. 1958: Genetic and Environmental Components of the Productivity of Perennial Ryegrass. *N.Z. J. agric. Res. 1:* 86-103.

Frandsen, K. J. 1956: Progeny Testing Methods. *Proc. 7th int. Grassl. Congr:* 415-26.

Frankel, O. 1935: Analytical Yield Investigations on New Zealand Wheat. II. Five Years of Analytical Variety Trials. *J. agric. Sci. 25:* 466-509.

Gotoh, K. 1956: Genetic Analysis of Varietal Differentiation in Cereals. III. Competitive Ability of Local Strains of the Barley Variety Hosogara 2. *Jap. J. Genet. 31:* 1-7.

Grissom, D. B.; Kalton, R. R. 1956: Inheritance of Combining Ability for Forage Traits in Bromegrass (*Bromus inermis* Leyss). *Agron. J. 48:* 289-93.

Gustafsson, A. 1953: The Co-operation of Genotypes in Barley-*Hereditas 39:* 1-18.

———1954: Mutation, Viability and Population Structure. *Acta agric. scand. IV:* 601-32.

Hodgson, G. L.; Blackman, G. E. 1956: An Analysis of the Influence of Plant Density on the Growth of *Vicia faba.* I. The Influence of Density on the Pattern of Development. *J. exp. Bot. 7:* 148-65.

———1957: An Anaylsis of the Influence of Plant Density on the Growth of *Vicia faba.* II. The Significance of Competition for Light in Relation to Plant Development at Different Densities. *Ibid. 8:* 195-219.

Keller, W. 1946: Design and Technic for the Adaptation of Controlled Competition to Forage Plant Breeding. *J. Amer. Soc. Agron. 38:* 580-88.

Kley, Van Der 1955: The Efficiency of Some Selection Methods in Populations Resulting from Crosses between Self-fertilising Plants. *Euphytica 4:* 58-66.

Lazenby, E. 1957: The Problem of Assessing Strains—A Study in Grass Breeding Technique. *J. agric. Sci. 48:* 294-304.

McDonald, E. D.; Kalton, R. R.; Weiss, M. G. 1952: Inter-relationships and Relative Variability Among S1 and Open-pollinated Progenies of Selected Bromegrass Clones. *Agron. J. 44:* 20-55.

Mather, K. 1955: Response to Selection. *Cold Spr. Harb. Symp. quant. Biol. 20:* 158-60.

Mitchell, K. J.; Coles, S. T. J. 1955: Effects of Defoliation and Shading on Short-Rotation Ryegrass. *N.Z. J. Sci. Tech. A36:* 586-604.

Murphy, R. P. 1952: Comparison of Different Types of Progeny in Evaluating Breeding Behaviour. *Proc. 6th int. Grassl. Congr.* 320-6.

Palmer, T. P. 1952: Population and Selection Studies in a *Triticum* Cross. *Heredity 6:* 171-85.

Sakai, K. 1951: Studies on Individual Selection and Selective Efficiency in Plant Breeding: *Jap. J. Breed. 1:* 1-9.

— 1953: Studies on Competition in Plants. I. Analysis of Competitional Variance in Mixed Plants Populations. *Jap. J. Bot. 14:* 161-68.

———1955: Competition in Plants and its Relation to Selection. *Cold Spr. Harb. Symp. quant. Biol. 20:* 137-57.

Sakai, K.; Gotoh, K. 1955: Studies in Competition in Plants. IV. Competitive Ability of F1 Hybrids in Barley. *J. Hered. 46:* 139-43.

Sakai, K.; Suzuki, Y. 1954: Studies on Competition in Plants. III. Competition and Spacing in One Dimension. *Jap. J. Genet. 29:* 197-201.

Sears, P. D. 1951: The Technique of Pasture Measurement. *N.Z. J. Sci. Tech. A33:* 1-29.

Suneson, C. A. 1949: Survival of Four Barley Varieties in a Mixture. *Agron. J. 41:* 459.

———1956: An Evolutionary Plant Breeding Method. *Ibid. 48:* 188-91.

Turesson, G. 1922: The Genotypical Response of the Plant Species to the Habitat. *Hereditas 3:* 211.

Whitehouse, R. N. 1953: Breeding for Yield in the Cereals. *Heredity 7:* 146.

BIOLOGICAL CONTROL FACTORS INFLUENCING POPULATIONS OF OAK LEAF-MINER, *Lithocolletis messaniella* Zeller, IN NEW ZEALAND INCLUDING THE INTRODUCTION OF PARASITES

By B. B. Given, Entomology Division, Department of Scientific and Industrial Research, Nelson.

(*Received for publication, 7 August 1958*)

Summary

The oak leaf-miner, a European species, was first recorded in New Zealand in February 1951. Populations of this insect in New Zealand suffer heavy mortality at certain times due to adverse food-plant leaf conditions. *Apanteles circumscriptus* Nees and *Enaysma splendens* Delucchi were selected from the European parasite complex for consignment to New Zealand and *Apanteles* material was sent during March and April and *Enaysma* during October 1957. During the summer of 1957-58 *Apanteles* reached high population levels in Nelson.

Introduction

The first record of *Lithocolletis messaniella* in New Zealand was from Gisborne in February 1951 (Wise 1953a). Spread was rapid and the pest now occurs throughout almost all areas where suitable food plants occur. During seasons of extreme abundance the food plant range (Wise 1953b, 1954, 1955) has extended beyond the normal limits as recorded in Europe and Asia and the fact that this over-flow population can survive on unusual food-plants points to a possible if unlikely danger. The fact that mines have been noted on at least one species of native beech (Wise 1953a) represents a potential danger to our indigenous forests. These facts, in addition to the desirability of retaining exotic oaks, beeches, chestnuts, etc. in their usual attractive condition prompted some action towards control of the pest.

Chemical control of pests on trees such as oaks under the conditions of distribution occurring in New Zealand is impracticable. Therefore, it was considered that the only hope of successful control would be by biological means. The prospects for success in this project were considered to be good since the miner is always at a very low population level in the areas of its origin (Europe and Asia)—indicating probable natural control—the larvae are readily accessible to parasites or predators, and the pest having no diapause presents no problem of synchronisation when introducing parasites from northern to southern hemispheres.

Life History and Population Level of the Pest in New Zealand

The miner in New Zealand appears to pass through six to seven generations per year, the length of the complete life-cycle, during January and February, being about 32 days at its minimum.

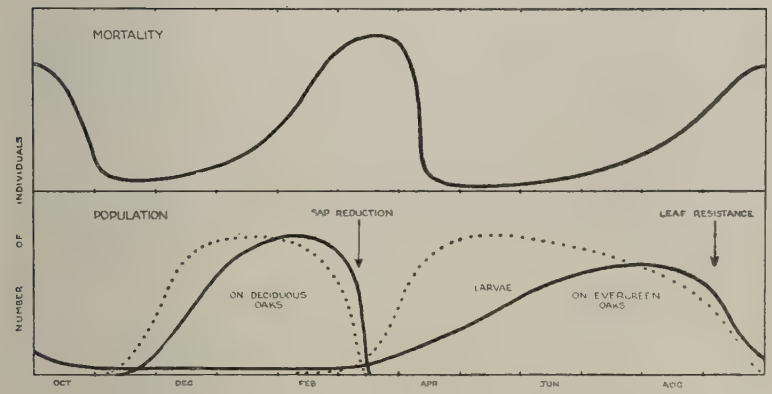


Fig. 1.—An approximation of seasonal population and mortality fluctuations of *Lithocolletis* in Nelson.

The seasonal population cycle shows two well defined peaks, each followed by a sudden drop which is in turn followed by a build-up which increases rapidly as the next peak is approached. The general seasonal population cycle is illustrated in Fig. 1. These graphs are designed to illustrate probable trends and are quantitatively purely arbitrary. The summer peak of population (principally on deciduous oaks) is at its maximum when quite suddenly, apparently coincident with cessation of sap flow, mortality rises to very high levels particularly

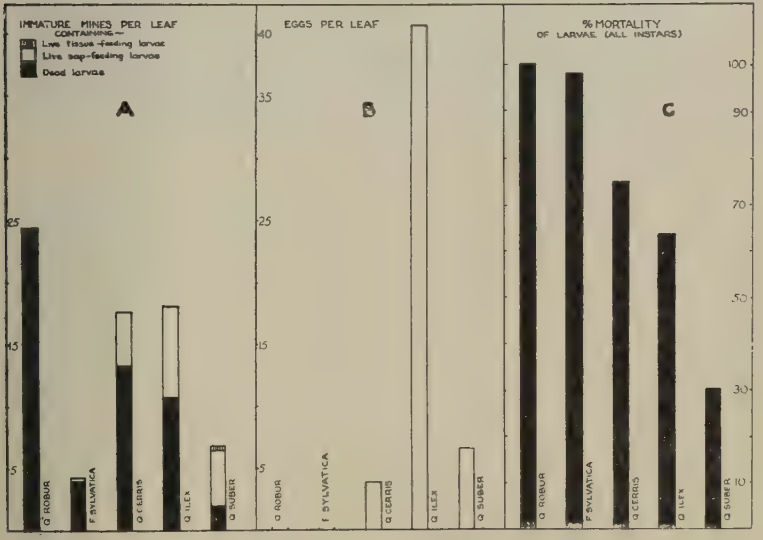


Fig. 2.—The status of *Lithocolletis* in Nelson on 22 April 1958. (Samples of ten mature leaves each.)

on *Quercus robur*. This is illustrated in more detail in Fig. 2 (A and C). It would appear that after the end of March, no larvae survive on *Q. robur*. It is of interest to note that although leaf fall is no later on *Q. cerris*, the mortality of larvae late in April is only about 73% compared with 100% in *Q. robur*. However, none of the remaining 27% of larvae on *Q. cerris* at this stage can survive.

Coincident with the onset of this high mortality rate in deciduous leaves is a transfer of oviposition to evergreen food-plants. From figure 2B it will be seen that oviposition in late April occurs on three of the five food plants investigated. Oviposition has ceased on *Q. robur* and *Fagus sylvatica* but eggs are still laid on *Q. cerris* although only in small numbers. At this stage, there is no hope of larval survival on *Q. cerris* beyond the first instar, which fact seems significant in oviposition response. The only obvious character present on leaves of *Q. cerris* and other selected leaves, e.g. *Q. suber, Q. ilex* and *Loropetalum chinense*) but not on rejected leaves (e.g. *Q. robur* and *F. sylvatica*) is lower surface hairiness. All the selected autumn food-plants studied have stellate hairs on the lower leaf-surfaces and, further, the density of oviposition is directly proportional to density of hairs. Further support is given to the hypothesis that hairs are a primary factor influencing autumn oviposition, by the fact that, of plants selected, *Loropetalum* alone has hairs on both leaf surfaces, and on this plant alone oviposition occurs on both surfaces.

It would appear then that there is a rather sudden and complete change in oviposition response from smooth to hairy leaves in the autumn. The spring change over to smooth leaves is not quite so complete, a few eggs being deposited on evergreen leaves throughout the summer.

The extreme mortality in late summer seems to indicate a lack of perfect synchronisation between the miner and its food plants in New Zealand. However, despite the enormous losses at this period, ample eggs are laid on evergreen trees to ensure the ultimate success of the winter generation.

In Fig. 1, the mortality and related population drop in spring and early summer are not quite as sharply represented as are the late summer curves. This is due largely to the fact that on *Q. suber* the drop is not so marked as on *Q. ilex*. Indeed, *Q. suber* is the only species studied that appears capable of carrying a continuous population of miner throughout the year. This spring population decrease is due, at least in Nelson, to leaf resistance in the case of *Q. ilex*, and principally to leaf-drying in the case of *Q. suber*. The leaf resistance set up by *Q. ilex* takes the form of parenchymatous proliferation within the mine ultimately squeezing the larvae to such an extent that insufficient space is left for movement and defecation. All larvae then die prior to hypermetamorphosis from sap- to tissue-feeding type.

Figure 3 is inserted to give some standard of comparison on a quantitative basis, of populations of miner prior to parasite introduction, with similar figures accumulated in future as parasite influence (if present) becomes felt. It is unfortunate that a more complete set

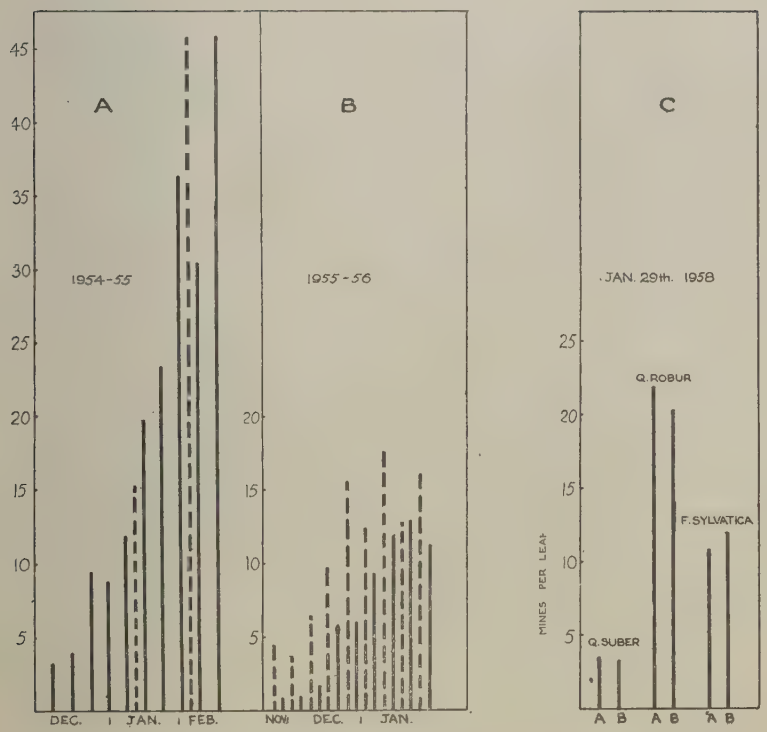


*Fig. 3.—Populations based on total mines per leaf. A and B, relative levels for 1954–55 and 1955–56 on *Q. robur*. Broken lines, Church Hill, Nelson; continuous lines, Queen's Gardens, Nelson. C, relative levels for *Q. suber, Q. robur,* and *Fagus sylvatica* in 1958. A and B (1954-55 and 1955-56) based on samples each of 20 leaves. C based on duplicated samples (A, B) of 80 leaves each except for *Q. suber* in which case samples consisted of 258 and 314 leaves.

of figures is not available to cover the pre-parasite liberation period, but if parasite influence is satisfactory, very definite reduction in these figures will be evident in future seasons.

The influence of population and mortality fluctuation of the host or parasite population and survival is of great importance, but is very difficult to assess accurately. At present we have not sufficient data to attempt such an assessment and must therefore confine our remarks to the general characteristic of the complex. It is obvious that during periods of high pre-adult host mortality, parasite losses must be of a correspondingly high order. Fortunately the parasite already established, *Apanteles circumscriptus* Nees, does not confine its late-summer

* All figures prior to 1957-58 were accumulated by Miss M. Stringer who worked on this project during the summer seasons concerned.

activities to the high larval populations on deciduous trees, but continues to operate on host material on *Q. suber* at low population levels. It would appear that the late summer and autumn period is the critical one as far as parasite success is concerned. There seems little doubt that parasite survival is assured, but whether or not the parasite can keep pace with and overtake the host population may be in some doubt.

Factors influencing host survival (and impinging on parasite survival) have been already mentioned on broad lines. An attempt was made to estimate the chances of survival from figures taken at the peak period of summer population. At this period, mortality is negligible in material that has reached the stage of producing tentiform (mature) mines, but will be very high in earlier stages.

On *Q. robur* the most frequently occurring number of mature or tentiform mines per leaf (Fig. 4) is between ten and fourteen and on *Q. suber* (Fig. 5) about two. In the absence of parasites, this would mean a probable population of approximately ten million moths per mature tree of *Q. robur* and four and a half million moths per mature tree of *Q. suber* during February. Moths may be expected to survive and oviposit for about four weeks. Most of this oviposition is on deciduous trees and will therefore be wasted, the commencement of mass oviposition on evergreens apparently occurring during March. It would therefore seem probable that not only are most of the larvae in early-stage mines in late January and early February doomed, but also the eggs produced by adults emerging at that time will be wasted. It therefore seems probable that it is the few adults ovipositing on evergreen species, particularly *Q. suber,* even as early as the beginning of February, that ensure survival of the colony.

What then, is the position where suitable evergreen food plants are said to be absent? Infestations appear to arise in early summer in such areas in New Zealand, just as readily as where evergreen oaks are present. Is this due to wind-carriage of moths or is there a small diapausing nucleus in fallen leaves? No concrete evidence of this latter condition has been obtained so far although thousands of fallen leaves have been examined. However, drifts of moths leaving heavily infested deciduous oaks on strong and even moderate winds have been seen, and it is possible that similar drifting occurs from evergreen species in early summer. The parasite so far established (*Apanteles circumscriptus*) has no diapause and appears to depend on winter populations of host material for year-round survival. It may therefore be necessary to establish trees of *Q. suber* in certain areas in order to ensure survival of this parasite.

Introduction and Establishment of *Apanteles circumscriptus*

After a careful survey of the parasite complex in Europe, the Commonwealth Institute of Biological Control selected two species for introduction into New Zealand. These were *Apanteles circumscriptus* Nees and *Enaysma splendens* Delucchi. In Europe it appears that *Apanteles* is a winter parasite while *Enaysma* is active only during the summer.

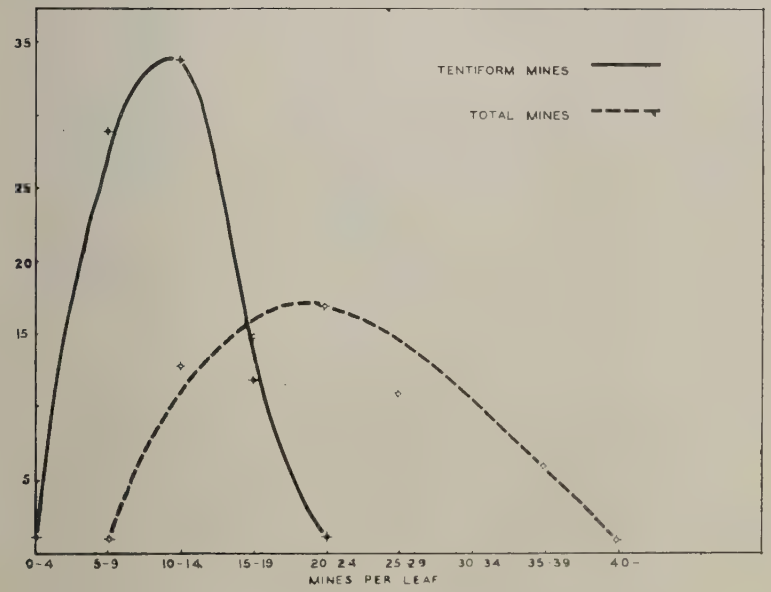


Fig. 4.—Frequency distribution of mines per leaf on *Q. robur* at 29 January 1958. Total number of leaves 80.

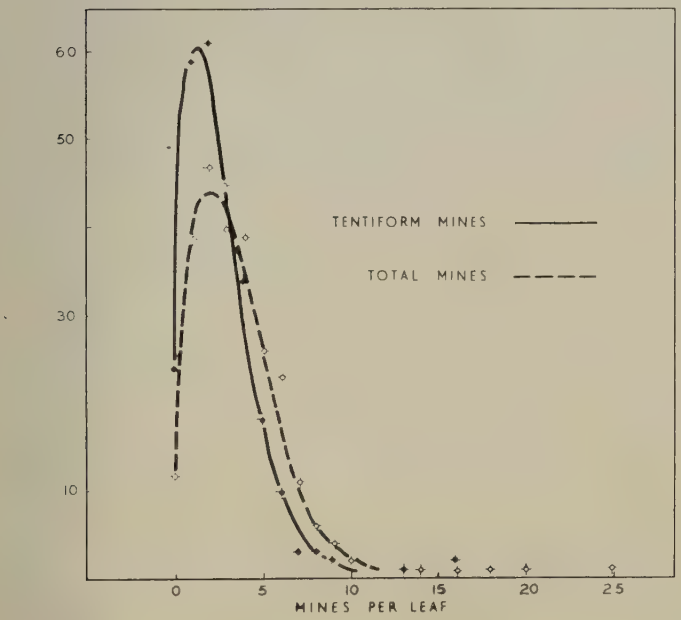


Fig. 5.—Frequency distribution of mines per leaf on *Q. suber* at 29 January 1958. Total number of leaves 258.

In Table 1, details of consignments of *A. circumscriptus* are given:

TABLE 1. CONSIGNMENTS OF *A. circumscriptus*

Consignment No.	Origin	Date Sent	Date Received	Cocoons	*Apanteles* emerging	
					Male	Female
1	Italy	22.3.57	28.3.57	54	2	40
2	Italy	2.4.57	8.4.57	500	2	444
3	Italy	8.4.57	15.4.57	400	1	322
				954	5	806

In addition to *Apanteles,* 32 hyperparasites and competing species emerged. These were immediately destroyed.

The method of liberation was by caging parasites on leaf clusters on local evergreen oaks which carried a good infestation of miner. A total of 42 cages was set up, each containing from ten to fifteen female *Apanteles.* After four or five days, cages were removed. By this time a moderate amount of oviposition had been achieved, the risks of multiparasitism due to confinement was minimised and the females were still able to parasitise suitable larvae away from the cage area. Parasitism was checked by removal of host larvae, mounting in Berlese's fluid and examination after clearing. From these examinations it was found that first-instar larvae were not parasitised, but in several cases practically all second-instar larvae were attacked. Percentages of total larvae parasitised ranged from 0% to over 50% in cage-samples tested.

One cage set up contained unmated female *Apanteles* as it was suspected that the species was parthenogenetic. Examination of three leaf samples disclosed percentages of parasitism of 38, 40, and 50. It is of interest that when males were confined with females, no mating or sexual interest of any description was noted. On this basis it was assumed that parthenogenetic reproduction was the rule and thereafter the lack of sufficient males was considered to be of no importance.

As a further test of effective parasitism by the use of the cage liberation method, three caged leaf clusters were placed in emergence containers in the laboratory and emergence recorded. Percentages of *Apanteles* to total emergence of *Apanteles* plus moths were 26, 9 and 36.

During the winter and spring of 1957, leaf samples were periodically taken and persistence of parasitism with slow saturation and spread noted.

With the onset of summer, on deciduous oaks, a parasite which has apparently found its own way into New Zealand but which is inactive during the winter months, begins to appear. This parasite is a species of *Pnigalio* in the opinion of Dr. Delucchi of the Commonwealth Institute of Biological Control, identical with a European species.

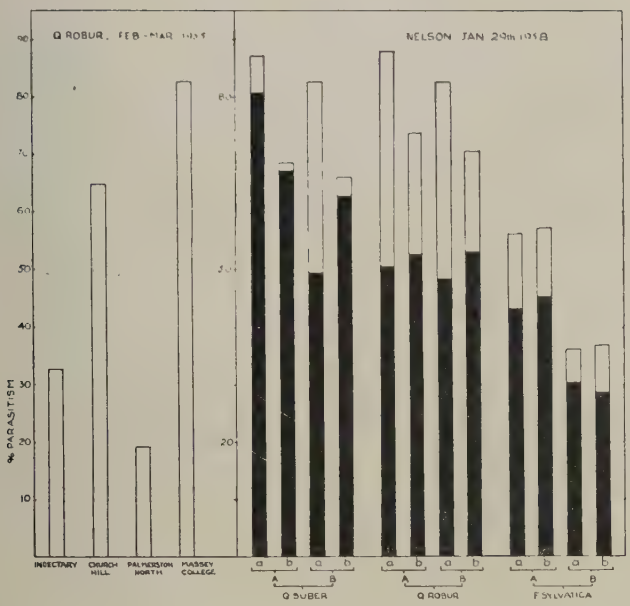


Fig. 6.—Percentages of parasitism before and after deliberate introduction of *A. circumscriptus*. White columns represent parasitism by *Pnigalio* sp., black columns represent parasitism by *Apanteles*. 1955 results are all based on emergence figures. Figures for 1955 are based on samples of emergents numbering not less than 200 moths plus parasites. 1958 figures are based on emergence (column a) of not less than 200 moths plus parasites and dissections (column b) of not less than 850 mines. In 1958 results, columns marked "a" are based on emergence, those marked "b" are based on dissections of mature mines. 1958 figures are all on paired samples, each pair marked "A" and "B".

Leaf samples of *Q. robur* collected on 29 January 1958 showed the presence of both *Pnigalio* and *Apanteles* (Fig. 6).

It is difficult to find an accurate method of determination of actual percentage of parasitism apart from dissection or slide-mounting of mature living larvae. The simplest method is to base counts on emergence figures, but in this method, unless the time of emergence of both moths and parasites does not exactly synchronise, an untrue figure will probably result. This would be levelled out by taking weekly samples but would not be practicable for wide-scale use. Dissection of mature mines has proved to be extremely laborious and the evidence of the dissected mine does not always allow of reliable interpretation. However, a comparison of these two methods (emergence counts and counts based on mine dissections) is illustrated in Fig. 6. It appears on comparative evidence from 1955 and 1958 figures that the presence of *Apanteles* has suppressed parasitism by *Pnigalio*. Future surveys should give further evidence in this respect.

From Fig. 6 it seems evident that the population level of *Apanteles* assisted by *Pnigalio* during the summer peak is sufficient to depress miner populations. However, the population drop following on this peak is so drastic as to be a very disturbing factor and it may well be that the host will bridge this mortality period better than will *Apanteles*, in which case *Apanteles* must overtake host populations once again during the winter months. *Pnigalio* is not to the same extent influenced as it enters diapause (pupal) about the time of onset of late-summer host mortality.

Introduction of *Enaysma splendens*

Although *E. splendens* has been liberated in the Nelson area, there is as yet no indication of establishment and it is not expected that any such indications will be noted before December 1958.

TABLE 2. Consignments of *E splendens*

Consignment No.	Origin	Date Sent	Date Received	Cocoons	*Enaysma* emerging
1	Switzerland	21.10.57	30.10.57	300+	183
2	Switzerland	29.10.57	5.11.57	326	169
				626+	352

In addition to *Enaysma,* 193 individuals of other species emerged and were immediately destroyed.

Practically all live *Enaysma* adults were liberated, mostly on *Q. cerris*. Only six liberation cages were set up, these utilising twenty-eight *Enaysma* adults. From two of these cages leaves were taken for checking of parasitism by emergence. In one case parasitism was 42% of total emergence of 43 moths plus parasites and in the other case results were negative.

The tree on which most of this parasite material was liberated is situated on one of the Nelson City council reserves. Normally the fallen leaves would be gathered for composting and later sterilising but the Superintendent of Parks and Reserves very kindly arranged to have the fallen leaves (in which *Enaysma* if present would be in diapause) transported to a new plantation of oaks and there distributed beneath the trees and left undisturbed. It will be in this area that surveys to check on establishment will be commenced during the summer of 1958-59.

Conclusion

While the establishment of *A. circumscriptus* has been very satisfactory in the Nelson district, the influence of the late-summer host mortality gives some cause for concern when considering ultimate control. Outside of Nelson, the prospects for general control and even parasite establishment in some areas must remain in doubt when the unsatisfactory distribution of evergreen food plants is considered.

It is possible that the selected summer parasite, *E. splendens* may be more effective in New Zealand as it is said to have a winter diapause which, if commenced sufficiently early and extending sufficiently long, could circumvent the need for evergreen oaks and could also cover by diapause the period of high late-summer mortality.

Taking all factors into consideration, although the promise of success with this project is good, it is by no means assured. The problem is one which presents many extremely interesting facets requiring fundamental investigation which from a purely scientific outlook would be very rewarding.

REFERENCES

Wise, K. A. J. 1953a: Occurrence of the Oak Blotch Miner *Lithocolletis messaniella* Zeller (Lepidoptera: Gracillariidae) in New Zealand. *Trans. roy. Soc. N.Z. 81:* 65-6.

————1953b: Host Plants of *Lithocolletis messaniella* Zeller (Lepidoptera: Gracillariidae) in New Zealand. *N.Z. J. Sci. Tech. A35:* 172-4.

————1954: Records and Observations of New Zealand Lepidoptera. *N.Z. Ent. 1 (4):* 27-30.

————1955: Notes on New Zealand Lepidoptera. *Ibid. 1 (5):* 13.

HIGH MOLYBDENUM INTAKE AND THE THRIFT OF YOUNG SHEEP

By I. J. Cunningham and K. G. Hogan, Animal Research Station, Department of Agriculture, Wallaceville

(*Received for publication, 23 July 1958*)

Summary

Two groups of newly weaned sheep were grazed for 12 months on pasture which for one group provided less than 1 p.p.m. molybdenum, and for the other 8 p.p.m. molybdenum. Copper content was around 7 p.p.m. and inorganic sulphate content averaged 0.4% for the first 7 months and 0.2% for the last 5 months. There was no adverse effect on growth or haematology from the high molybdenum. In conjunction with high molybdenum, the higher inorganic sulphate prevented copper storage, but copper storage did occur when inorganic sulphate was at the lower level. Molybdenum appeared in the blood at lower but not at the higher level of inorganic sulphate. High molybdenum in the diet caused accumulation of molybdenum in bones, kidney, and spleen. It is concluded that high molybdenum in the diet is not itself toxic and that it is harmful to sheep only when there is also present sufficient inorganic sulphate and a low enough dietary copper for copper depletion and copper deficiency symptoms to occur.

Introduction

A number of soil types in New Zealand, covering some millions of acres, produce pasture that has more than the normal content of molybdenum (Cunningham *et al.* 1956), and cattle that are reared on many of these soils show signs of molybdenosis and induced copper deficiency, which respond to treatment with copper. This reaction of cattle to extra dietary molybdenum occurs with fairly considerable regularity, and there are not many instances when a pasture high in molybdenum does not cause evidence of disease in grazing cattle. The position with sheep is by no means so uniform. On some farms, where cattle are seriously affected by molybdenosis, sheep have shown no ill effect; on other farms in different areas sheep do show evidence of disease and may do this even on pastures that have lower molybdenum contents. The symptoms are those associated with deficiency of copper, e.g. ataxia in the lambs, loss of crimp in merino wool, or fragility of bones in lambs. In all cases these symptoms have been corrected by increasing the supply of copper.

Scouring and unthriftiness in lambs, which are corrected by administration of copper, have been reported from one area of marine silt where the pastures are high in molybdenum (Byrne and Collin 1955). These symptoms do not occur with a simple deficiency of copper and would therefore appear not to result from copper depletion alone but to be the result of molybdenum poisoning in sheep.

This apparent specific reaction of young sheep to molybdenum, together with the lack of conformance in the occurrence of disease in sheep and cattle on other areas where the pasture is high in molybdenum, suggested the need to make detailed observations on the growth and

thrift of sheep grazed on pasture containing high levels of molybdenum. This paper reports the results of such work.

EXPERIMENTAL

Two groups of lambs were grazed from weaning, one group on pasture of normal molybdenum content, the other on pasture of which the molybdenum content had been increased by topdressing with molybdate.

Pastures. The paddocks carried a good sward, dominantly perennial ryegrass and white clover, which had been sown down for 5 years and had been adequately topdressed with superphosphate and lime. Superphosphate had been applied at 3 cwt per acre each year and carbonate of lime at 1 ton per acre on two occasions, one 5 years and the other 4 years before the present experiment started. Cobalt sulphate at 5 oz per acre was topdressed over all paddocks at the commencement of the experiment as the area was mildly cobalt-deficient.

The control group was rotated over three paddocks, each approximately ⅔ acre. The molybdenum group, which contained twice as many animals as the control group, was handled as two subgroups and each subgroup rotated over 3 paddocks each of approximately ⅔ acre. Thus the conditions of husbandry in groups 1 and 2 were the same, despite the difference in size of the groups.

Molybdate topdressings were applied as a spray of a solution of ammonium molybdate in water. Six ounces ammonium molybdate per acre were applied two months before the lambs commenced grazing the experimental paddocks. A further topdressing was applied on 5 November 1956, and on this occasion 4 oz ammonium molybdate per acre was used on some paddocks and 8 oz per acre on others in an endeavour to get as uniform as possible an increase in the molybdenum content of the pasture. The average values each month for the copper, molybdenum, and inorganic sulphate in the pastures available to the two experimental groups are shown in Table 2.

Animals. These were derived from 3 sources. The animals shown in the tables with origin marked A were from ewes that had been running continuously for 3 years on pasture topdressed to raise the molybdenum content to around 6–8 p.p.m. Animals of origin B were from a paddock adjacent to A, which had no molybdate topdressing, and on which the pasture had normal low levels of molybdenum. Animals of group C came from Silverstream, where molybdenum content of pasture was also low.

The composition of pasture on which A, B, and C animals were reared to weaner age is shown in Table 1.

The animals were transferred to the experimental groups shortly after weaning and regular observations were made, over a period of 12 months, of body weight, blood copper and molybdenum, haemoglobin, packed cell volume and red blood cell concentration. Wool weights were recorded, copper and molybdenum were determined in liver biopsy samples on one occasion, and copper and molybdenum were also deter-

mined in some of the tissues of wether animals after slaughter at the end of the experiment.

Copper was determined by the method described by Clare *et al.* (1945); molybdenum, except in bone, by Perrin's (1946) method; inorganic sulphate by the method of Johnson and Nishita (1952), and blood counts by conventional methods. The method used for molybdenum in bone involved preliminary dry ashing, separation of molybdenum as cupferrate in chloroform, removal of chloroform and treatment then as from the beginning in Perrin's method.

Results

Pasture Composition

Pre-weaning Period. The composition of the pasture available to the lambs of origin A, B, and C during the pre-weaning period is shown in Table 1.

TABLE 1. Composition of Pastures on which Lambs Reared (D.M. basis)

Origin of Lambs	Date Sampled 1955	Cu p.p.m.	Mo p.p.m.	SO_4 %
A	Sep.	7.6	9.6	0.30
	Oct.	7.0	8.6	0.30
	Nov.	5.3	5.4	
B	Sep.	6.5	0.9	0.39
	Oct.	6.2	0.5	0.32
	Nov.	5.2	0.5	0.35
C	Oct.	8.9	2.1	0.23

The figures show that A lambs, in addition to being born from mothers that had grazed on grass of high molybdenum content for 3 years, themselves ate high-molybdenum grass in the pre-weaning period. The grass was somewhat low in copper content.

B lambs had grass that was also similarly rather low in copper, but normal in molybdenum.

C lambs had grass that was normal in copper and molybdenum contents.

Experimental Paddocks. The average values for copper, molybdenum, and inorganic sulphate in samples taken each month of the grass available to groups 1 and 2 is shown in Table 2.

Group 1 sheep had pasture that was adequate in copper content though a little lower than is usually found in New Zealand pastures, and had a normal low content of molybdenum. The pastures of group 2 sheep were similar in copper and inorganic sulphate contents but

TABLE 2. COPPER, MOLYBDENUM AND SULPHATE CONTENTS
(Pasture D.M.)

Date	Group 1			Group 2		
	Cu p.p.m.	Mo p.p.m.	SO_4 %	Cu p.p.m.	Mo p.p.m.	SO_4 %
19.1.56	8.5	0.9	0.33	8.7	13.8	0.34
8.3.56	7.6	0.5	0.64	6.9	12.6	0.56
3.4.56	8.1	0.5	0.54	7.7	6.6	0.58
2.5.56	10.0	1.2	0.49	8.9	9.0	0.49
11.6.56	8.7	0.5	0.36	7.9	6.7	0.41
19.7.56	7.8	0.5	0.27	7.1	4.9	0.31
20.8.56	7.2	1.3	0.25	6.8	9.0	0.24
7.9.56	7.4	0.5	0.16	7.0	7.0	0.20
9.10.56	9.2	0.5	0.22	8.0	5.9	0.17
7.11.56	7.8	0.5	0.22	7.5	22.3	0.22
22.11.56	7.0	0.5	0.17	7.2	11.0	0.26
4.12.56	6.2	0.5	0.19	6.1	10.8	0.17
18.12.56	5.2	0.5	0.17	5.7	12.0	0.19
22.1.57	4.8	0.8	0.18	4.0	6.7	0.17

much higher in molybdenum. The only difference in the intake of these three consituents by the two groups of experimental sheep was that group 2 sheep ate grass with an average of about 8 p.p.m. of molybdenum, whereas group 1 sheep ate grass with less than 1 p.p.m. of molybdenum.

The inorganic sulphate content was much higher for the period January to August 1956 than for September onwards. The average for the earlier period was 0.42% or enough to provide at least 4 g inorganic

TABLE 3. BODY WEIGHTS AND WOOL WEIGHTS
(lb)

			Body Weight												Wool Weight
Group	Origin	No.	Feb. 1956	Mar. 1956	Apr. 1956	May 1956	June 1956	July 1956	Aug. 1956	Sept. 1956	Oct. 1956	Nov. 1956	Dec. 1956	Jan. 1957	Sept. 1956
1	A	5	65	70	75	77	81	86	84	82	94	100	110	114	9.0
	B	9	67	75	80	80	81	83	78	75	84	89	98	102	7.9
	Av.	14	66	73	78	79	81	84	81	78	87	93	102	107	8.3
2	A	9	66	75	75	78	74	79	78	78	86	90	101	102	8.3
	B	2	71	78	71	74	72	76	73	75	85	91	102	100	8.2
	C	18	66	73	72	76	74	79	76	76	83	86	98	97	9.2
	Av.	29	67	74	73	77	74	79	76	77	84	88	99	99	8.8

sulphate daily to each sheep. From the second period the average content was 0.20%, or enough to supply at least 2 g per sheep daily.

Growth and Wool

Table 3 shows the body weights each month and the weight of wool shorn in September. In each group the average for sheep of the same origin is given as well as the average for all animals in the group.

There was no appreciable difference in the growth of the two groups. The slightly lower weights in group 2 animals were probably the result of an earlier infestation of *Haemonchus contortus,* which was brought on to the experimental area by the C sheep. All animals were treated with phenothiazine, but the infestation was not entirely cleared up in the earlier months, and, in June, group 2 animals showed over twice the number of eggs per gramme of faeces (av. 4,200 e/g) as were present in faeces of group 1 lambs. After another treatment with phenothiazine at this time there was no further evidence of worm infestation.

There was no difference in the average weights of wool shorn from the two groups.

Copper and Molybdenum in Blood

The results of the examination of bloods taken at intervals of about two months are shown in Table 4.

Blood copper was low in A lambs at the commencement, at intermediate levels in B lambs, and normal in C lambs. Values rose quickly to normal in all groups after the start of the experiment and remained normal throughout.

A lambs had small amounts of molybdenum in the blood at the start no doubt because of the high molybdenum in the pasture on which they were reared. The A lambs in group 1 quickly lost the blood molybdenum and only traces of molybdenum were subsequently detected in animals of this group. Group 2 animals, on the other hand, showed appreciable amounts of molybdenum in the blood from August onwards. The appearance of molybdenum in the blood at this time was probably associated with a lower inorganic sulphate content in the pasture at the time.

Haematology

The values for haemoglobin, packed cell volume, and red blood cells in samples taken on five occasions are shown in Table 5.

Group 2 animals showed slightly lower values for all three constituents, especially in June, and it is possible that the low figures at this time were the result of a mild haemonchosis. The lower figures for the whole group in other early months were due mostly to low values for C sheep right from the start. The blood picture of these sheep improved in the course of the experiment to become closely similar to that for other members of the same group and for group 1 sheep.

TABLE 4. COPPER AND MOLYBDENUM IN BLOOD
(μg/ml)

Group	Origin	No.	Feb. 1956		Mar. 1956		June 1956		Aug. 1956		Oct. 1956		Nov. 1956		Jan. 1957	
			Cu	Mo	Cu	Mo	Cu	Mo	Cu	Mo	Cu	Mo	Cu	Mo	Cu	Mo
1	A	5	0.040	0.011	0.085	*	0.086	*	0.090	*	0.105	*	0.102	*	0.084	*
	B	9	0.061	0.004	0.083	*	0.085	*	0.091	*	0.101	*	0.097	*	0.083	*
	Av.	14	0.053	0.007	0.084	*	0.085	*	0.091	*	0.103	*	0.102	*	0.084	*
2	A	9	0.027	0.020	0.085	*	0.056	*	0.048	0.027	0.100	0.122	0.096	0.182	0.081	0.094
	B	2	0.065	0.008	0.098	*	0.058	*	0.056	0.011	0.070	0.125	0.076	0.043	0.074	0.135
	C	18	0.085	*	0.103	*	0.082	*	0.083	0.013	0.091	0.093	0.088	0.095	0.076	0.119
	Av.	29	0.065	0.018	0.096	*	0.072	*	0.070	0.017	0.094	0.101	0.090	0.118	0.078	0.112

TABLE 5. BLOOD—HAEMOGLOBIN, PACKED CELL VOLUME AND RED BLOOD CORPUSCLES

Date	Group 1					Group 2				
	Origin	No.	Hb %	PCV %	Rbc 10^6/mm^3	Origin	No.	Hb %	PCV %	Rbc 10^6/mm^3
Feb. 1956	A	5	11.4	45	10.8	A	9	11.2	41	11.0
	B	9	11.4	45	11.3	B	2	11.4	44	11.0
						C	18	10.0	36.2	10.0
	Av.	14	11.4	45	11.1	Av.	29	10.4	38.2	10.4
May 1956	A	5	9.8	40	11.4	A	9	11.0	45	11.8
	B	9	11.3	40	11.8	B	2	12.3	46	11.5
						C	18	9.3	35.9	9.4
	Av.	14	10.6	40	11.6	Av.	29	10.0	39.4	10.3
June 1956	A	5	12.1	50	12.2	A	9	10.0	35	11.7
	B	9	11.6	45	11.8	B	2	9.7	36	11.2
						C	18	9.3	34	10.9
	Av.	14	11.8	47	12.0	Av.	29	9.5	34.4	11.2
Aug. 1956	A	5	13.9	46	11.7	A	9	13.4	42	10.9
	B	9	13.5	46	11.1	B	2	13.9	43	11.1
						C	18	12.7	40.6	10.2
	Av.	14	13.7	46	11.3	Av.	29	13.0	41.2	10.5
Oct. 1956	A	5	13.0	39	11.3	A	9	12.8	41	11.0
	B	9	12.9	39	11.2	B	2	13.4	46	11.6
						C	18	12.9	41.7	11.2
	Av.	14	12.9	39	11.3	Av.	29	12.9	41.7	11.1

Copper and Molybdenum in Liver Biopsy Samples

One biopsy of liver was taken in September 1956 after the animals had been on experiment for 8 months. The results for copper and molybdenum are given in Table 6.

The copper values were distinctly lower and the molybdenum values higher in group 2 livers, whatever the origin of the sheep. The very low copper values for A and B sheep in group 2 did not cause any adverse effect that was measured in the examinations made.

Copper and Molybdenum in Tissues

The wether animals, of which there were 5 in group 1 and 7 in group 2, were slaughtered at the end of the experiment and the average values for copper and molybdenum in liver, kidney, spleen, and bone are shown in Table 7.

TABLE 6. COPPER AND MOLYBDENUM IN LIVER BIOPSY SAMPLES
(p.p.m. in dry liver)

Group	Origin	No.	September 1956	
			Cu	Mo
1	A	5	101	6.7
	B	9	156	6.0
	Av.	14	144	6.4
2	A	9	16	11.2
	B	2	16	10.7
	C	18	65	12.3
	Av.	29	46	11.8

TABLE 7. COPPER AND MOLYBDENUM IN TISSUES TAKEN AT AUTOPSY
(p.p.m. in dry tissue)

Group	Origin	No.	Bone	Liver		Kidney		Spleen	
			Mo	Cu	Mo	Cu	Mo	Cu	Mo
1	A	2	*	315	3.0	13.0	1.2	4.5	*
	B	3	*	422	1.8	12.9	3.3	4.5	*
	Av.	5	*	380	2.2	12.9	2.4	4.5	*
2	A	5	9.5	133	7.6	16.5	15.4	4.5	9.8
	B	2	11.0	39	8.1	13.1	14.7	4.7	10.3
	Av.	7	9.9	106	7.7	15.5	15.3	4.6	9.9

* = less than 1 p.p.m.

In specimens from group 2 animals there were high concentrations of molybdenum in bone, kidney, and spleen, and somewhat less in liver. Specimens from group 1 animals contained smaller amounts of molybdenum in liver and kidney, and only traces in bone and spleen.

There was a difference in copper contents between the groups only for the livers. There had, however, been a very considerbale increase in both groups over the values found in the biopsy liver samples taken 4 months earlier.

Discussion

The purpose was to determine the effect of increased dietary molybdenum, and therefore copper and inorganic sulphate as well as molybdenum were measured in the pastures fed to the experimental sheep because these three substances, as Dick (1953) has shown, must be considered together in metabolism of the sheep.

The copper content of the pastures available to both groups was the same and was adequate to meet the copper requirement, provided there was no interference from other substances in the diet. Results for group 1 sheep, which had a normal molybdenum intake, showed that copper supplies had, in fact, been adequate, since blood copper, copper in liver biopsy samples, and copper in tissues taken at autopsy, were all within the range found by experience to be normal for sheep.

The inorganic sulphate content of the pastures varied considerably, and there was a definite difference in the values for the first half of the feeding period from those in the second part. The average of the monthly values for January to August was 0.42% for each group, and that from September to the following January was 0.20%. There were thus two distinct periods of different inorganic sulphate supply. In the first 7 months the sheep ingested about 4 g inorganic sulphate per day, whereas in the next 5 months the average daily intake was about 2 g per day. These intakes were the same for both groups.

The molybdenum contents in the pastures were distinctly different for the two groups. Group 1 pasture contained around 1 p.p.m. or less, which is a level that is found in many normal good sheep pastures. Group 2 pastures varied from 5 to over 13 p.p.m. molybdenum at different times, but provided an average level of 8 p.p.m. throughout the experiment and the same average for each of the two periods separated out on account of difference in inorganic sulphate content of the herbage.

There were variations in copper and inorganic sulphate inherent in any diet dependent on grazed pasture, but these variations affected both groups equally and the significant difference between the groups was the higher molybdenum content of pastures available to group 2 animals. This pasture, which provided an average level of 8 p.p.m. molybdenum, had no adverse effect on young growing sheep that was disclosed in the observations made. Body weight, wool growth, blood copper, haemoglobin, packed cell volume, and red blood cell concentration were closely similar in sheep ingesting this level of molybdenum and in control sheep receiving less than 1 p.p.m. molybdenum in the diet. It is apparent that this level of molybdenum was not itself harmful when fed to sheep over a period of 12 months after weaning. Furthermore, some of the sheep (of origin A) were exposed to an even longer period of high molybdenum intake, since they were born and reared on pasture with a similar high molybdenum content. No special effect was noted in these sheep in comparison with their group mates reared on a normal diet of molybdenum.

The tolerance of these experimental sheep of a high molybdenum intake, together with field observations that sheep live on some high-

molybdenum areas with no apparent adverse effects, agree in showing that molybdenum itself is not severely toxic to sheep.

There is, however, an apparent discrepancy, for on some high-molybdenum areas where molybdenum content of the pasture is no higher than in this experiment, symptoms of disease due to copper deficiency do occur in sheep. Since the symptoms associated with molybdenum excess are those of copper deficiency and since it has been shown that there is no evidence of direct toxicity of molybdenum, it would appear that high molybdenum in pasture is likely to be harmful only when conditions are right for the molybdenum to induce a pathological copper deficiency in grazing sheep. Amongst such conditions are a low intake of copper and, as has been shown by Dick (1954), a high intake of inorganic sulphate.

Some information on the level of inorganic sulphate in a pasture diet that is necessary for loss of copper in sheep is given in the present experiment, from a consideration of the low copper contents of the liver biopsy samples taken in September and the much higher copper contents of whole livers taken at autopsy 4 months later. The liver biopsy samples were taken after a period of 8 months during which the pasture diet contained 0.4% inorganic sulphate, and the whole livers were obtained after a further period of 4 months during which the pasture contained 0.2% inorganic sulphate. The molybdenum was similar over the two periods and the copper was somewhat lower in the second period. It is not unreasonable to suppose, therefore, that storage of copper in the second period was the result of there being insufficient inorganic sulphate to restrict this storage.

That would suggest that loss of copper in grazing sheep is not likely to occur from high dietary molybdenum unless the content of inorganic sulphate in the pasture is above 0.2% and probably in the region of 0.4% or about 4 g daily per sheep of about 70 lb live weight. Such a figure agrees with Dick's (1954) findings on sheep pen-fed dry rations of somewhat similar copper and molybdenum contents. Loss of copper was also shown by Wynne and McClymont (1956) in pen-fed sheep on dry rations containing 0.4% of inorganic sulphate. The loss of copper observed by them was much higher than in the present experiment, even though the molybdenum content of their diet was smaller: the copper content of their diet was also lower and it is likely that it was this lower copper intake which allowed a greater loss of copper. That would accord with field observations, which show the exacerbated effect of high molybdenum intake on copper loss when copper supplies are low, and also with the observed control of loss of copper by increasing copper intake through topdressing pastures with copper salts, or by direct administration of copper to animals.

The present work has shown that a level of inorganic sulphate in the pasture greater than 0.2% is needed along with increased molybdenum to cause loss of copper in grazing sheep. For cattle a much lower level of inorganic sulphate was found effective as no increased excretion of copper could be induced by increasing the inorganic sulphate above 0.1% in dry rations (Cunningham *et al.* 1958). This difference in the reaction of sheep and cattle to inorganic sulphate

and molybdenum may explain some of the differences in the metabolism of copper in these two species. It has, for example, been found in practice that cattle are much more sensitive to excess pasture molybdenum than are sheep, and it may be that it is on the areas where inorganic sulphate is lower that cattle become copper deficient though sheep do not. This possibility is at present being examined. At this stage, it is known that there is considerable variation in the inorganic sulphate content of pastures on high-molybdenum areas. The average content of some 73 such samples so far examined is 0.36%, and the range 0.09% to 0.85%. For 32 samples from areas where sheep are known to be affected, the average inorganic sulphate is 0.42% and for 41 from areas where sheep are not affected, or where the history in that respect is incomplete, the average is 0.30%. Again sheep are known to accumulate copper very much more readily than cattle. It may be that this happens because the higher dietary inorganic sulphate required to control copper storage in the sheep is less frequently available than is the lower level needed by cattle.

In addition to the effect the variation of inorganic sulphate in the pasture had on the storage of copper, there was a clear difference in molybdenum storage between the period of high and low inorganic sulphate intakes. In group 2, blood molybdenum was present in only trace quantities up to August, i.e. for the period when inorganic sulphate content of the pasture was 0.4%. After this, when the inorganic sulphate dropped to 0.2% in the pasture, molybdenum appeared in the blood of group 2 sheep. The presence of molybdenum in the blood in sheep on a high molybdenum intake indicates that there is insufficient dietary inorganic sulphate to promote excretion of all the molybdenum and probably indicates also that there is insufficient to prevent storage of copper.

The high concentration of molybdenum in the tissues of sheep on high molybdenum intake, especially the high values for bone, kidney, and spleen, is of some value as a post-mortem diagnostic test for exposure to high molybdenum.

References

Byrne, J. J.; Collin, F. H. 1955: *Proc. 17th Conf. N.Z. Grassl. Ass.* pp. 128-38.

Clare, N. T.; Cunningham, I. J.; Perrin, D. D. 1945: *N.Z. J. Sci. Tech. A26:* 340-50.

Cunningham, I. J.; Hogan, K. G.; Breen, J. N. 1956: *Ibid. A38:* 225-38.

Cunningham, I. J.; Hogan, K. G.; Lawson, B. M. 1958: *N.Z. J. agric. Res. 2:* 145-52.

Dick, A. T. 1953: *Aust. vet. J. 29:* 233.

———1954: *Aust. J. agric. Res. 5:* 511.

Johnson, C. M.; Nishita, H. 1952: *Anal. Chem. 24:* 736.

Perrin, D. D. 1946: *N.Z. J. Sci. Tech. A27:* 396-405.

Wynne, K. N.; McClymont, G. L. 1956: *Aust. J. agric. Res. 7:* 45.

THE EFFECT OF SULPHATE AND MOLYBDENUM ON COPPER METABOLISM IN CATTLE

By I. J. Cunningham, K. G. Hogan, and B. M. Lawson, Animal Research Station, Department of Agriculture, Wallaceville

(*Received for publication, 15 July 1958*)

Summary

Jersey cattle were fed from yearling stage for 2 years on a ration low in copper and molybdenum, and containing 0.1% of inorganic sulphate; supplements of these substances were dosed to different groups.

Additional molybdenum increased blood and liver molybdenum and decreased blood and liver copper. Inorganic sulphate in addition to molybdenum prevented accumulation of molybdenum in blood or liver, but did not increase the loss of copper from blood or liver. It was concluded that any interaction there may be between molybdenum and sulphate on copper was provided for by the 0.1% inorganic sulphate in the basal ration. It is suggested that the sensitivity of cattle to high molybdenum is related to the low level of accompanying inorganic sulphate that is necessary to cause loss of copper.

Introduction

It has been shown by Dick (1953) that molybdenum and inorganic sulphate in the diet limit the absorption and storage of copper by the sheep. This is an important contribution to the understanding of the metabolism of copper and may well be important also to the understanding of some of the diseases associated with disturbances of copper supply, that occur in the grazing sheep.

Cattle are even more sensitive than sheep to high contents of molybdenum in the pasture that they graze, and it is therefore of even greater importance to know whether in this species also inorganic sulphate plays any part in influencing the action of molybdenum on copper metabolism. Mylrea (1958) has now shown that there is a molybdenum-sulphate interaction with copper in bovines; our own experiments on this question are described in this paper.

Experimental

Five groups of four Jersey heifers were fed a basal ration of meadow hay, oat grain, and calcium carbonate, supplemented by 0.5 g α-tocopherol per animal once a month. The 20 animals were run together in a small enclosure and feeding commenced on 3 August 1954. The daily ration of crushed oat grain was increased from 30 to 80 lb for the group in the course of the first two weeks and was kept at the latter figure throughout the experiment. One half-pound of precipitated chalk was added to each 80 lb of oat grain. Hay feeding commenced at 200 lb per day for the group and this was raised to 400 lb per day in 5 weeks and kept at that level for the rest of the experiment.

Supplements of copper, molybdenum, and sulphate were given to the animals in the different groups by drenching, on 5 days a week,

TABLE 1. Chemical Analysis of Hay and Oats
(on D.M.)

	p.p.m.		per cent			
	Cu	Mo	Inorganic SO_4	CaO	P_2O_5	Crude Protein
Meadow hay	4.0	0.47	0.1	0.6	0.3	9.1
Oat grain	3.0	0.35	0.03	0.07	0.6	6.7

solutions of copper sulphate, ammonium molybdate, or sodium sulphate. The equivalent daily intake of copper, molybdenum, and inorganic sulphate provided by the drenches are shown in Table 2, which also shows the composition of the diet of each of the five groups when allowance is made for the intake in the basal ration and in the drenches.

TABLE 2. Supplements for Each Group and Resultant Composition of the Diet

Group	Drench Equivalent daily dose			Composition of diet (on D.M.)		
	Cu (mg)	Mo (mg)	SO_4 (g)	Cu (p.p.m.)	Mo (p.p.m.)	Inorganic SO_4 (%)
1	0	0	0	4.0	0.5	0.1
2	0	169	0	4.0	16.0	0.1
3	54	169	0	9.0	16.0	0.1
4	0	169	51	4.0	16.0	0.55
5	54	169	51	9.0	16.0	0.55

Dosing of the supplements commenced on 30 August 1954 at which time the animals were approximately one year old, and continued until 3 July 1956, after which other treatments were given to some of the animals. Regular observations were made of body weights, dry-matter content of faeces, copper and molybdenum contents of liver biopsy samples and blood samples, and haemoglobin content, red-cell count and packed cell volume of blood. Inorganic sulphate determinations were made on the blood on one occasion.

Copper was determined by the method described by Clare *et al.* (1945), molybdenum by Perrin's (1946) method, except in the case of the low values in fodders, and inorganic sulphate by the method of Johnson and Nishita (1952). For low molybdenum concentrations, 50 to 100 g material was dry ashed, the ash (including residue after removal of any silica with hydrofluoric acid) dissolved in acid, molybdenum separated as cupferrate in chloroform, chloroform removed and residue treated as from the beginning in Perrin's method. Other determinations were made by conventional methods in everyday use at the Station.

RESULTS

Results are shown in Table 3. When the experiment had been in progress a short while it was found that one heifer in each of group 2 and 4 was pregnant and these calved after 3 months feeding. On two occasions later on in the experiment a young bull gained access to the group and the result was that two animals in group 1 calved in October 1955 and two in September 1956. Calves were removed at birth. These accidental variations to the planned experiment would be expected to have some effect on the body weights and possibly some effect on the composition of the liver.

Body Weights

There was a steady but slow increase in body weights in all groups and the low liver and blood copper values for groups 2 and 4 were not accompanied by any slower rate of growth. The slow growth indicated that the ration was capable of providing only slightly above maintenance requirement. The lower final weights reached by group 1 animals were probably associated with the drain of bearing a calf on a poor ration.

Liver Copper and Molybdenum

The small sample that is obtained on biopsy was satisfactory for copper determination, but was rather small for any certainty about the figure obtained for molybdenum. The molybdenum values however were consistent and therefore are included; they show that the supplements of molybdenum raised the molybdenum in the liver but when supplements of inorganic sulphate also were given this rise did not occur.

Liver copper showed a steady fall in all groups, which was arrested to some extent by the supplements of copper given to groups 3 and 5. The supplement of molybdenum in group 2 and those of molybdenum and inorganic sulphate in group 4 caused a marked depletion of the liver copper; this was no more marked in group 4 than in group 2 despite the extra inorganic sulphate supplied to group 4. Again the extra inorganic sulphate supplied to group 5 did not cause a greater depletion of liver copper than group 3, for which the diet was the same except for the sulphate.

Faeces Dry Matter

These measurements were made to have a measure of the degree of diarrhoea should this occur in the groups receiving extra molybdenum. No diarrhoea was noted clinically and the dry-matter contents of the faeces indicate that their consistency was the same in all groups.

Blood Copper and Molybdenum

Molybdenum was found in appreciable amounts in groups 2 and 3, to which groups supplements of molybdenum without sulphate were given. In groups 4 and 5, which received the same amount of molybdenum but had additional inorganic sulphate, the blood molybdenum was much lower and was closely similar to the levels in group 1, which received no supplementary molybdenum.

TABLE 3. EXPERIMENTAL DATA

Body Weight (lb)

Group	Supplements	Aug. 1954	Oct. 1954	Nov. 1954	Dec. 1954	Feb. 1955	Apr. 1955
1	Nil	433	460	504	500		603
2	Mo	449	475	479	506		623
3	Mo, Cu	461	506	550	553		671
4	Mo, SO_4	469	486	527	540		614
5	Cu, Mo SO_4	431	456	497	526		623

Liver Biopsy. Copper and Molybdenum (p.p.m.)

		Cu	Cu	Mo	Cu	Mo	Cu	Mo	Cu	Cu	Mo
1	Nil	130	123	2.8	104	*	60	7.1	46	31	2.3
2	Mo	143	107	9.7	61	13.0	39	13.7	24	20	10.4
3	Mo, Cu	125	185	10.9	130	17.2	82	14.3	110	92	9.6
4	Mo, SO_4	167	131	3.3	89	6.0	64	7.0	33	22	3.4
5	Cu, Mo SO_4	282	181	3.5	191	10.3	177	9.3	202	202	4.8

Faeces Dry Matter (%)

1	Nil	22.4	16.0	13.2			
2	Mo	19.2	16.2	13.1			
3	Mo, Cu	17.8	15.9	14.3			
4	Mo, SO_4	19.4	13.6	13.5			
5	Cu, Mo SO_4	18.8	16.2	14.4			

Blood Cu and Mo (mg/100 ml)

		Cu	Mo			Cu	Mo	Cu	Mo	Cu	Mo
1	Nil	.092	*			.100	*	.108	.033	0.93	.007
2	Mo	.099	*			.114	.221	.105	.078	.085	.125
3	Mo, Cu	.102	.14			.104	.190	.102	.068	.100	.150
4	Mo, SO_4	.103	*			.122	.026	.101	.031	.090	.005
5	Cu, Mo SO_4	.106	*			.125	.031	.116	.029	.100	.010

Haemoglobin (g%)

1	Nil	10.7				10.3	
2	Mo	11.2				10.3	
3	Mo, Cu	10.6				10.2	
4	Mo, SO_4	10.4				10.2	
5	Cu, Mo SO_4	9.5				9.9	

* = trace.

June 1955	Aug. 1955	Oct. 1955	Dec. 1955	Feb. 1956	Mar. 1956	May 1956	July 1956
668		706		701	691		683
711		791		854	867		858
721		812		871	878		873
670		723		784	791		773
681		737		782	775		782

Cu	Mo	Cu	Mo	Cu	Mo	Cu	Mo	Cu	Mo	Cu	Mo	Cu
56	3.9	73	5.2	72	5.5	91	6.5	78	9.8	25	4.2	25
14	13.9	12	20.1	9	11.0	10	8.0	10	9.4	9	9.2	9
123	13.9	152	19.0	150	9.2	106	8.5	94	9.8	76	6.9	50
17	5.2	15	5.6	12.5	5.7	11	5.5	11	7.6	8.1	4.0	9
128	5.8	175	7.2	167	5.3	158	6.9	161	8.0	128	5.1	81

12.3
14.5
11.4
10.2
13.5

Cu	Mo	Cu	Mo	Cu	Cu	Mo	Cu	Mo	Cu	Mo
.096	.016	.090	.013	.095	.081	.002	.071	.027	.063	.012
.087	.250	.070	.380	.059	.056	.103	.055	.178	.046	.043
.106	.232	.098	.358	.102	.100	.114	.099	.135	.094	.037
.100	.029	.073	.021	.076	.067	.009	.065	.019	.052	.025
.113	.038	.106	.031	.098	.105	.014	.099	.019	.097	.017

10.0	9.5
11.0	11.0
11.4	11.4
11.8	11.1
11.1	11.0

Blood copper dropped somewhat in the control group and more in groups 2 and 4, which received molybdenum. There was no greater drop in group 4, which received inorganic sulphate as well as molybdenum. The supplements of copper for groups 3 and 5 kept the blood copper approximately at normal levels.

Haemoglobin

There was no significant difference in haemoglobin values between the groups on any of the sampling dates. On the same dates determinations were made of red blood cells and packed cell volumes, but these were normal and there were no variations between animals or groups and consequently the figures are not reported in detail.

Inorganic Sulphate in Blood

Determinations made on July 1956 gave results that were closely similar in all groups. The average values in microgrammes inorganic sulphate per millilitre whole blood for groups 1 to 5 were respectively 96, 96, 111, 105, and 108.

Discussion

A surprising feature was the absence of scouring in cows that were ingesting a diet containing 16 p.p.m. molybdenum and especially in animals of groups 2 and 4, in which liver copper contents were very low. High dietary molybdenum is an invariable part of the history of peat scours (Cunningham 1946) in which disease, as the name indicates, scouring is a notable symptom. Moreover, experiments with cattle on a peat soil that grew pastures low in copper and low also in molybdenum have shown that increasing the intake of molybdenum did induce scouring (Cunningham 1950). Further experience with high dietary molybdenum for cattle on some pumice areas has shown that a high intake of molybdenum, even when associated with an induced copper deficiency, does not necessarily result in scouring (Cunningham 1957). That experience was confirmed in the experiment described above. Some other factors in association with high molybdenum and low copper must play a part in causing scouring. Inorganic sulphate, because of its possible interaction with molybdenum and copper, might be thought of as having such activity, but the results obtained show that this is not the case. Mylrea (1958), moreover, mentions no scour-inducing effect of inorganic sulphate, even when 1.2% of inorganic sulphate was added to a diet high in molybdenum. Mylrea further added 300 p.p.m. of manganese to make the content of his ration 391 p.p.m. but scouring was not recorded from this addition. At the end of the experimental period in the present work some of the cows with low liver copper and high molybdenum intake were grazed for a period each day on lush pastures, but this did not induce scouring. Others on high molybdenum intake were drenched daily for 23 days with 200 g sodium chloride while eating lush pasture, but this again did not cause scouring. It would appear that the scour-inducing factor postulated was not present in the pasture fed, nor in any of the additions mentioned.

The supplements of molybdenum did have some definite effects. Molybdenum, alone or with copper, caused considerable increases in

the concentration of molybdenum present in the liver and the blood. When extra inorganic sulphate was fed along with the molybdenum these increases did not occur.

Molybdenum, alone or with inorganic sulphate, caused a decrease in the copper content of the liver and of the blood. There was no greater effect from molybdenum and inorganic sulphate than from molybdenum alone. Mylrea (1958) did find an enhanced effect on copper excretion when inorganic sulphate was added to a diet containing 0.03% sulphate. The absence of any enhancement of effect in our work suggests that the maximal sulphate effect was produced by the 0.1% inorganic sulphate in the basal ration.

Though no additional excretion of copper was produced by increasing the inorganic sulphate content of this diet, there was a definite effect from extra sulphate of preventing the accumulation of molybdenum in the blood and tissues. Thus there was demonstrated a clear difference in bovines between the effects of dietary inorganic sulphate on the storage of molybdenum and on the storage of copper.

A question of interest and importance is what part sulphate may play in the metabolism of copper and molybdenum by animals on the high-molybdenum pastures of this country. Inorganic sulphate determinations are in the process of being carried out on pasture samples collected from a large number of different soil types. Two classes may be briefly mentioned at this time—the samples from land normal in molybdenum content and samples from high-molybdenum pastures. Of 92 samples in the first group, the average inorganic sulphate content is 0.35%, and the range 0.09% to 1.01%; in 45 high-molybdenum pastures the average is 0.33%, and the range 0.09% to 0.85%. In each class there was one sample in which the result was below 0.1%.

The inorganic sulphate contents were closely similar in these two classes of pasture, and in only one of 45 high-molybdenum pastures was the content below 0.1%. In all except one of the high-molybdenum pastures there was therefore enough inorganic sulphate for the high molybdenum to exert its maximal effect in restricting the storage of, or causing excretion of, copper. Thus, in practically all places where a molybdenum excess occurs, an induced copper deficiency can be expected in cattle.

The sensitivity of cattle to excess molybdenum in the pasture is no doubt related to the fact that copper is lost at a level of inorganic sulphate likely to be present in all pastures (except swards that are practically pure clover) that have been topdressed with superphosphate.

In those pastures with an inorganic sulphate content near 0.1% there might, on the evidence of the present work, be too little sulphate to prevent some accumulation of molybdenum in blood or tissues. In agreement with this it has not been uncommon to find moybdenum present in tissues from animals on high-molybdenum pastures. It seems not unlikely that in such cases there might be interference with the functioning of copper in processes such as pigmentation, in addition to the induced deficiency of this element.

References

Clare, N. T.; Cunningham, I. J.; Perrin, D. D. 1945: *N.Z. J. Sci. Tech. A26:* 340-50.

Cunningham, I. J. 1946: *N.Z. J. Sci. Tech. A27:* 381.

——————1950: In W. D. McElroy and B. Glass "Symposium on Copper Metabolism". p. 246. Johns Hopkins Univ. Baltimore.

——————1957: *N.Z. J. Agric. 95:* 218.

Dick, A. T. 1953: *Aust. vet. J. 29:* 233.

Johnson, C. M.; Nishita, H. 1952: *Anal. Chem. 24:* 736.

Mylrea, P. J. 1958: *Aust. J. agric. Res. 9:* 373-84.

Perrin, D. D. 1946: *N.Z. J. Sci. Tech. A27:* 396-405.

A NOTE ON FUNGI ISOLATED FROM STAINED WOOL

By H. Jacks, Massey Agricultural College, Palmerston North

Small pieces of wool showing grey and yellow to reddish-brown stains were washed in 7 changes of sterile distilled water, plated on Czapek Dox and potato dextrose agar, and incubated at 20°C.

A number of almost pure fungal colonies developed in the cultures. Intense pink-to-red, olive-green, yellow-to-brown, and grey-to-black pigments developed, these pigments darkening with age when viewed from the reverse side of cultures.

The causal organisms were tentatively identified as:

Phoma spp.
Chaetomium spp.
Epicoccum spp.
Macrosporium spp.

As the occurrence of stained wool (from which the sample originated) was limited to the lower portions of the fleece, it is assumed that the above soil fungi stained the wool when the sheep were lying down.

THE EFFECTS OF TOPDRESSING AND OVERSOWING HILL PASTURES

By F. E. T. Suckling, Grasslands Division, Department of Scientific and Industrial Research, Palmerston North

(*Received for publication, 28 July 1958*)

Summary

The results of a five-year field trial to measure the effects of superphosphate and surface sowing with clovers on hill pastures are presented. Clover establishment from surface sowing was recorded in permanent quadrats, and changes in botanical composition of the pasture were measured by the point analysis method. Herbage yields and composition were determined by frame cuts. By the end of the five-year period, oversowing alone had increased yield by 22%, fertiliser alone by 36% and seed and fertiliser together had doubled yield.

Introduction

The greater part of New Zealand hill country is extremely uneven in contour, botanical composition, soil moisture and climatic conditions. Pasture management practices vary accordingly. Wherever hill country is ploughable, ploughing not only destroys weeds and secondary growth, but also evens out the humps and hollows. This leads to more uniformity in moisture, soil fertility, and also stock grazing, and consequently a more uniform pasture association with a high production potential. However, the experiment described in this paper is concerned largely with unploughable, wet hill country which, by virtue of its uneven nature, always contains a variety of species with a wide range of productivity. On the hilltops, and on other areas with high fertility and uniform moisture supply, ryegrass will predominate, while on the dry knolls *Danthonia* and other low fertility species will persist. Because of this uneven pasture association, it is of interest to determine the possible improvement of the existing pasture by the introduction of appropriate legumes and the use of mineral fertiliser.

In a large-scale grazing trial at this station, comparisons had been made between untreated paddocks and those that were oversown and topdressed, on the assumption that oversowing alone or fertiliser alone would not produce maximum growth (Suckling 1954). The present experiment was a small paddock trial to compare pasture production from other combinations of these two additives. At the commencement of the experiment, the pasture was typical of run-out hill country to be seen throughout the North Island, with a carrying capacity of only about 1½ ewes per acre.

Experimental

Soil

The trials were laid down on an undulating saddle at the Te Awa hill-pasture research area some 30 miles north-east of Palmerston North. Soil type is classified as Raumai sandy loam 11H (N.Z. Soil Bureau 1954). Soil on the plot area was fairly uniform and had the following profile:

8 in. dark-greyish-brown fine sandy loam, friable, strongly developed fine nutty structure with a clear boundary;
4 in. light-grey sandy clay loam with many faint fine yellowish-brown mottlings and a few iron and manganese concretions overlying a light-grey sandy clay loam.

Climate

The area is approximately 1,000 ft above sea level and rainfall for the last ten years has averaged 42 in., evenly spread throughout the year, with occasional short summer droughts occurring during the January to March period. The lowest falls occur in March and September, with an average of 2 in. per month. The mean monthly rainfall is approximately 4½ in. and has been recorded in June, July, October and November.

Earth temperature recordings at 4 in. depth reach a monthly average of 65°F in January and drop to 43°F in July.

Layout and Procedure

In March 1950, a paddock-scale experiment was laid down in which there were four treatments in two randomised blocks. Each paddock was $\frac{1}{20}$ acre in area and the original pasture appeared to be fairly uniform and was predominantly browntop, Chewings fescue, Yorkshire fog, sweet vernal, with small amounts of ryegrass, cocksfoot, *Danthonia* and *Poa* species. White clover was sparse and a small quantity of suckling clover was present. Flat weeds and moss were plentiful (Table 3).

The four treatments applied were:—

O No seed or fertiliser—Control.
F Fertiliser—Superphosphate at 4 cwt per acre in autumn each year.
S Clover seed—Mixed clovers and *Lotus*.
F + S Fertiliser and seed.

The area was first hard grazed with wethers, after which the appropriate paddocks were surface sown with clovers and topdressed. These operations were completed on 24 March 1950 and two pasture measurement frames (10 × 4 ft) were placed on each of the 8 separately fenced paddocks. Superphosphate was applied at the rate of 4 cwt per acre annually for the five years and the seed mixture used was as follows:—

4 lb	subterranean clover (i.e. 2 lb Mt. Barker 2 lb Tallarook)
3 lb	white clover
3 lb	broad red clover
3 lb	Montgomery red clover
1 lb	lotus major (*Lotus uliginosus*)
14 lb	per acre

All seed used had a minimum germination of 95%.

To record the strike of oversown clover seed, eight 1-sq-ft quadrats were permanently pegged in each paddock. Counts of clover seedlings were made over the first year.

Dry ewes and wethers were used for grazing, which took place at approximately monthly intervals but more frequently during spring. Frame cuts coincided with the termination of grazing, and stocking rates were determined by the availability of feed. Herbage was cut by hand shears down to grazing height and the frame shifted to new locations at each grazing. Where necessary, frame sites were pre-trimmed to remove roughage. All paddocks were simultaneously stocked and sheep-days were recorded for each treatment.

RESULTS

Clover establishment counts on the oversown areas are shown in Table 1. Results with red and white clover were satisfactory, but there was a poorer strike of subterranean clover, while *Lotus* failed almost completely.

A summary of herbage production from the four treatments is shown in Fig. 1, while Table 2 gives dry matter yields, as well as botanical composition. A histogram (Fig. 2) shows species contribution to the overall annual production over the five-year period.

TABLE 1. CLOVER ESTABLISHMENT COUNTS
(Expressed as a percentage of seed sown)

Averages of 16 1-sq-foot quadrats

Treatment	Seeds Sown per Quadrat	19.4.50		9.5.50		4.7.50		25.9.50		5.2.51		Standard Error
		S	F+S	S	F+S	S	F+S	S	F+S	S	F+S	
Red	16	43	18	37	15	25	14	23	11	23	11	± 2
White	45	51	32	29	15	*						± 4
Sub.	6	20	24	17	15	18	12	15	11	2	3	± 5
Lotus	20	2	2	6	2	8	3	3	2	1	1	± 1

* Sown plants could not be identified after this date.

Changes in ground cover of the various species were recorded each season, using the point-analysis technique (Levy and Madden 1933). Analyses were made at the same time each year and in every case shortly after a paddock had been grazed and when the herbage height was 1½–2 in. Table 3 shows the general picture of the change in ground cover over the trial period, by comparing the position at the commencement of the trial with the average of all measurements throughout the five-year period and with the final analyses at the end of the trial. Table 4 shows the average of all the seasonal analyses for the two

TABLE 2. ANNUAL AND TOTAL HERBAGE PRODUCTION AND BOTANICAL COMPOSITION

Period	Treatment	Total D.M. lb/ac	Botanical Composition (% of dry matter)												
			Ryegrass	Cocksfoot	Dogstail	Chewings fescue	Browntop	Sweet vernal	Yorkshire fog	*Poa trivialis*	Red clover	White clover	Subterranean clover	Other grasses	Other species
1950/1	O	5500	12	2	13	4	15	17	16	2	—	5	—	1	13
" "	F	7300	15	2	10	5	14	17	16	3	—	12	—	Tr	6
" "	S	5500	14	2	12	2	12	16	15	1	3	6	1	Tr	16
" "	F+S	7400	18	Tr	13	4	7	15	12	1	2	11	2	Tr	15
1951/2	O	4600	7	1	13	11	15	13	17	2	—	6	—	1	14
" "	F	7300	16	2	13	6	11	11	19	4	—	13	—	1	4
" "	S	5500	7	2	12	6	15	14	14	3	7	7	1	1	11
" "	F+S	8100	13	Tr	11	9	11	7	13	5	5	16	1	1	8
1952/3	O	6200	9	1	7	12	15	15	23	2	—	5	—	Tr	11
" "	F	9900	8	5	7	13	12	10	27	4	—	7	—	1	6
" "	S	9400	13	4	8	4	11	11	29	4	5	4	Tr	1	6
" "	F+S	13300	15	1	5	6	12	11	24	13	2	4	Tr	Tr	7
1953/4	O	8100	9	Tr	10	10	8	17	18	1	—	4	—	1	22
" "	F	12500	15	3	8	12	8	11	18	6	—	10	—	2	7
" "	S	11900	11	2	11	9	7	10	16	3	4	6	9	2	10
" "	F+S	14800	13	1	11	7	6	11	18	7	7	11	3	2	3
1954/5	O	8900	14	2	7	12	10	13	26	2	—	4	—	1	9
" "	F	12100	19	7	3	13	10	6	23	3	—	8	—	2	6
" "	S	10900	19	8	6	6	7	6	20	4	5	8	1	2	8
" "	F+S	17700	28	5	2	7	5	5	24	7	3	9	Tr	2	3
S.E.'s		±1100	±2									±2.5			
Average annual yield 1950-55	O	6700	10	1	9	10	12	15	21	2	—	5	—	1	14
	F	9300	15	4	8	10	11	10	21	4	—	10	—	1	6
	S	8600	13	4	9	6	10	11	19	3	5	6	3	1	10
	F+S	12300	18	2	7	7	9	9	20	7	4	10	1	1	6

TABLE 3. BOTANICAL COMPOSITION RECORDED BY THE POINT ANALYSIS TECHNIQUE
(hits per 100 points examined)

Species		Start of Trial	Average of 5 years				End of Trial				S.E.'s
Common Name	Botanical Name	0	O	F	S	F+S	O	F	S	F+S	
Ryegrass	*Lolium perenne*	1	13	22	20	26	16	25	22	36	± 4
Cocksfoot	*Dactylis glomerata*	1	3	7	3	2	5	14	6	3	± 2
Dogstail	*Cynosurus cristatus*	22	27	27	28	30	12	7	11	3	± 4
Chewings fescue	*Festuca rubra commutata*	21	36	29	25	27	25	17	16	13	± 4
Danthonia	*Danthonia* spp.	5	6	5	6	2	7	2	5	—	± 2
Browntop	*Agrostis tenuis*	38	30	28	27	28	17	11	15	15	± 4
Sweet vernal	*Anthoxanthum odoratum*	39	25	24	27	24	6	10	9	10	± 2
Yorkshire fog	*Holcus lanatus*	11	25	26	26	25	24	36	29	33	± 4
Other grasses		2	4	7	4	8	5	6	5	8	± 2
Red clover	*Trifolium pratense*	—	—	—	12	12	—	—	15	10	± 2
White clover	*Trifolium repens*	9	24	36	36	52	25	40	28	44	± 4
Suckling clover	*Trifolium dubium*	2	2	1	1	Tr	1	1	1	—	± 0.5
Sub. clover	*Trifolium subterraneum*	—	—	—	7	5	—	—	5	1	± 2
Lotus	*Lotus uliginosus*	—	—	—	Tr	—	—	—	—	—	—
Catsear	*Hypochaeris radicata*	22	23	13	24	14	12	4	8	3	± 4
Other species		7	10	7	8	5	13	4	13	6	± 2
Bare ground		—	1	Tr	Tr	Tr	1	1	1	Tr	—

TABLE 4. SEASONAL CHANGES IN PASTURE COVER
(Five-year average of hits per 100 points examined each season throughout trial period)

	Control						Seed + Fertiliser					
	Ryegrass	Dogstail	Other grasses	White clover	Other clovers	Weeds	Ryegrass	Dogstail	Other grasses	White clover	Other clovers	Weeds
Autumn	18	29	123	21	1	34	32	32	104	46	12	17
Winter	9	29	123	14	2	31	22	34	111	45	14	12
Spring	9	26	154	22	2	46	19	27	130	55	20	24
Summer	17	22	124	31	2	44	29	20	118	59	17	24
S.E. ±	3	3	7	6	3	3	3	3	7	6	3	3

TABLE 5. PERCENTAGE PROTEIN CONTENT OF HERBAGE FOR THE VARIOUS TREATMENTS

Season	Five-year Average and Range Between Years			
	O	F	S	F + S
Autumn	16.5 13.8 — 19.6	17.9 15.3 — 21.3	19.1 16.1 — 24.5	19.5 17.4 — 21.7
Winter	17.6 14.4 — 19.9	18.9 15.2 — 21.9	19.3 13.0 — 23.8	19.8 16.6 — 22.7
Spring	17.7 17.5 — 18.0	18.4 17.2 — 20.1	18.9 18.0 — 19.6	20.3 18.2 — 21.2
Summer	14.2 12.1 — 16.3	15.0 12.8 — 16.7	15.0 13.3 — 16.9	16.3 13.6 — 18.6
Annual	16.5 14.7 — 17.9	17.5 15.5 — 19.2	18.1 16.2 — 19.9	19.0 17.5 — 20.5
S.E. of seasonal figures	± 0.4			
Annual average crude protein per acre (lb)	1110	1720	1560	2340
	S.E. ± 167			

TABLE 6. CHEMICAL ANALYSIS OF TOP TWO INCHES OF SOIL. SEPTEMBER 1955

Treatment	pH	1% Citric Acid Soluble P. mg %	C %	N %	C/N	Exch. Cap. m.e.%	Base Sat. %	Ca m.e.%	Mg m.e.%	K m.e.%
O	5.2	5	5.1	0.40	13	16.6	45	5.3	1.8	0.90
F	5.5	14	5.8	0.59	10	18.9	56	8.0	1.9	1.20
S	5.6	5	5.4	0.52	10	18.0	56	7.0	1.8	1.30
F+S	5.6	14	5.9	0.56	10	17.7	65	8.0	2.2	1.40

The methods used in these analyses are as described by Metson (1956).

extreme treatments to show the seasonal changes in ground cover of the various species.

Nitrogen analyses of the herbage from the plots were carried out on bulked samples from each frame cut (Table 5). Limited soil analyses were carried out by the Soil Bureau at the end of the trial (Table 6).

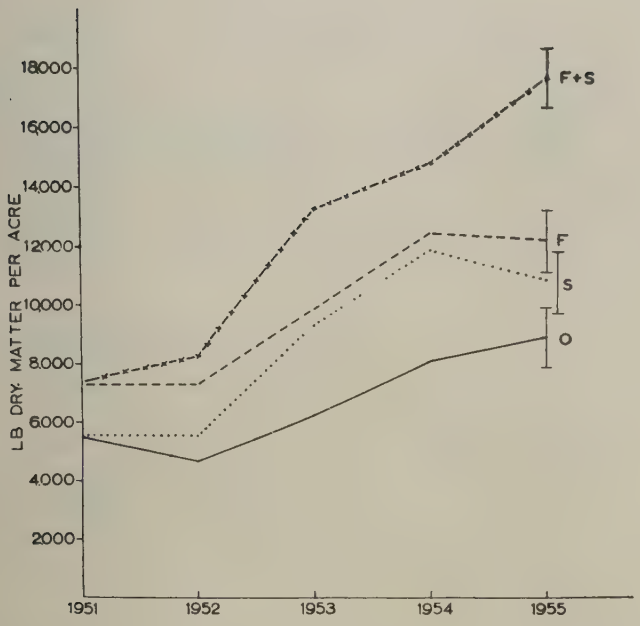


Fig. 1.—Average total production 1950-55 O = Control F = Fertiliser S = Clover seed F+S = Fertiliser + Clover seed. S.E.'s indicated by vertical bars.

DISCUSSION

Clover Establishment

Initial clover germination, and establishment following germination were high for red and white clover. However, for all species, in Table 1, the percentage establishment decreased with successive counts. White-clover seedlings were not counted after May because they could not be distinguished from white-clover plants already in the sward. Subterranean clover, being an annual, would normally set seed and die off in the summer. Hence it is reasonable to assume that the September count would approximate the number of plants likely to set seed. *Lotus uliginosus* was slow to establish and was unable to stand competition from other species, particularly where fertiliser was applied. Under conditions of fairly strong competition from grasses, and with the intermittent grazing practised, *Lotus* seedlings reached the second trifoliate leaf stage 12 months from sowing but were very small and etiolated. Subsequent botanical analyses over the following five years failed to show the presence of *Lotus*.

It is apparent that heavy superphosphate applications reduced the establishment rate of clover seedlings. This is probably due to competition. Levy (1951) emphasised the possibility of the early loss of clovers and slow-growing grasses when sown under vigorous cereal or cover crops. Sears (1950) reported differences in clover establish-

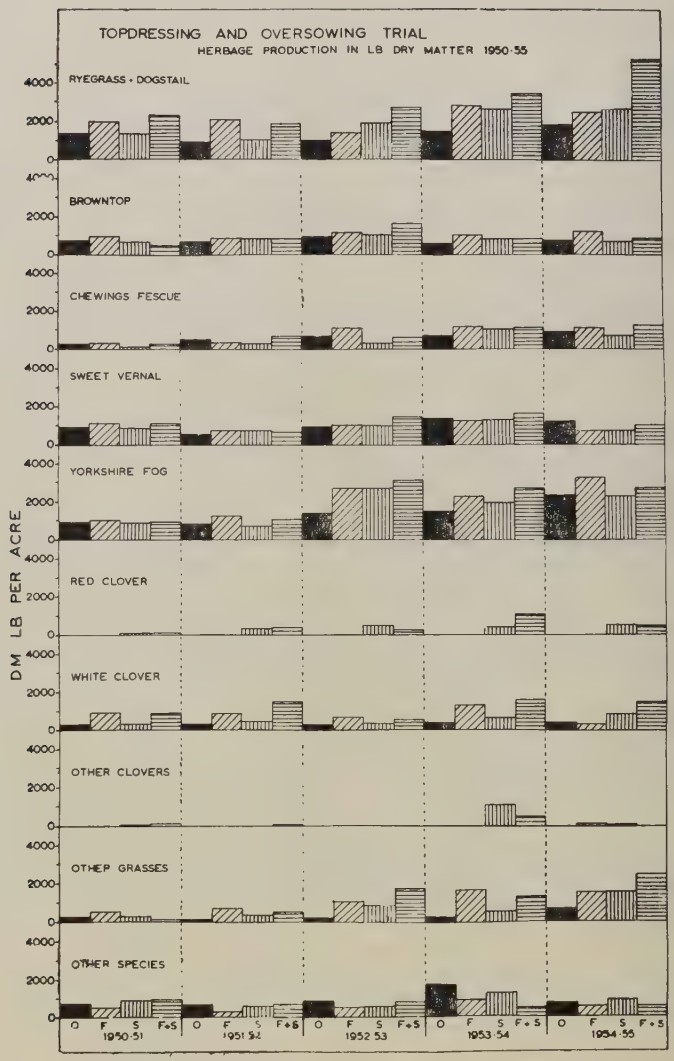


Fig. 2.—Herbage production in lb dry matter per acre for the years 1950-55.

ment in a new pasture containing perennial ryegrass, short-rotation ryegrass, and red and white clover sown on fertile land. Clover suppression was greatest under tall grass, while practically no suppression occurred when pasture was close grazed. Suckling (1951) obtained similar results with surface-sown clovers on hill pasture. Clover establishment on the present area could have been stronger with more frequent

grazing to suppress grass growth. However, with the heavy seeding rate used, a satisfactory clover population was achieved.

Herbage Production

The herbage production of the various treatments over the five-year period is shown in Fig. 1 and Table 2. The total herbage production of the oversown topdressed paddocks was superior in all five years to the other treatments. Fertiliser alone produced the next highest total production, with seed alone in third position and control showing the lowest figure.

Statistical analyses of the dry-matter production from the various treatments showed a year × treatment interaction significant at the 10% level. Fertiliser showed an increase in yield early in the trial, while oversown clover seed showed an effect later. By the final year, fertiliser + seed was easily the best treatment. On a percentage basis, oversowing and topdressing increased the dry-matter production to 199% of control, fertiliser alone to 136%, and seed alone to 122% in the fifth year of the trial.

Botanical Composition

Botanical composition was greatly influenced by the treatments. Throughout the five-year period the oversown and topdressed paddocks had vigorous clover and were dark green in colour. In the paddocks where clover alone was sown, there was a lack of vigour in the pasture although adequate clover established. Superphosphate, without oversowing, encouraged the existing white clover to grow more strongly although this clover was in fact of a poor-producing type (as determined by the picric acid test). The control paddocks appeared to lack vigour throughout the trial period, although it was apparent that the heavier-than-normal stocking improved all pasture and resulted in higher production. Stock management thus played an important part in building fertility and in utilising the additional herbage produced.

Details of the botanical changes, as determined by herbage dissection of each cut and then recalculated for annual yields are shown in Table 2. Changes in ground cover, as indicated by seasonal point analyses are shown in Table 3. However, a brief summary of the botanical changes in the four treatments over the five-year period is as follows:—

1. *Control.* Over the whole trial period, the control paddocks remained essentially similar to the surrounding unimproved pastures but with a slightly higher proportion of ryegrass and white clover. The main contributors were sweet vernal, Yorkshire fog, Chewings fescue, and flat weeds, with considerable moss showing in the bottom.

2. *Seed Alone.* The main difference between this treatment and the control was that there was a noticeable growth of red, white, and subterranean clovers. Although these oversown clovers established and persisted throughout, they appeared to be somewhat unthrifty. There was, however, an increase in ryegrass, Yorkshire fog, cocksfoot, and *Poa trivialis*. This, no doubt, was a result of the trebling of total clover content compared with control.

3. *Fertiliser Alone.* There was a rapid and marked increase in the growth of white clover and all the associated grasses, particularly ryegrass, Yorkshire fog, and *Poa trivialis.* A definite thickening of the sward resulted, with consequent reduction of flat weeds.

4. *Fertiliser and Seed.* There was a rapid increase in growth and vigour of all the established species similar to that seen in the fertilised area, but, in addition, the oversown red and white clovers established and grew strongly. Subterranean clover established in the first year but did not persist, presumably from its inability to re-establish in the tight sward of ryegrass, Yorkshire fog, *Poa trivialis,* and white and red clover. A notable feature of this pasture was the continued increase of *Poa trivialis* due, no doubt, to the extra growth between grazings, as well as the steady rise in soil fertility. Better control of this species would perhaps have been obtained if this treatment had been grazed more often particularly in the winter periods.

Table 4 shows a comparison between the control and the oversown and topdressed areas in terms of the ground covered by the various species at different times of the year. As can be seen, there was, in general, a complete ground cover throughout the year on both treatments. The effect of the seed and fertiliser treatment was to produce a denser pasture from the extra growth of white clover and ryegrass in place of flat weeds and some of the low-fertility grasses.

Between seasons, the most noticeable feature was the tendency for the pasture to become more open in the winter and early spring, when white clover and ryegrass growth were both at a relatively low ebb. This was compensated to some extent by the growth of *Poa trivialis.* It was noticeable, too, that the greatest growth of flat weeds occurred in the late spring and summer periods.

Crude Protein

No doubt as a result of the intensive grazing control, the general level of crude protein in the herbage from all treatments was relatively high. However, there were differences between treatments (Table 5) which serve not only as an index of rapidity of growth, but also as an index of palatability.

For all seasons, the control paddocks were lower in crude protein than those of the other three treatments. The five-year average shows the highest protein for the fertiliser + seed pastures, seed next, with fertiliser third, and control lowest. Herbage protein was consistently lower in the summer months than during the other three seasons, with high figures for both winter and spring, and an intermediate level for the autumn.

Statistical analysis of the protein percentage showed that there were significant effects due to both factors, viz oversowing and fertilising.

The protein-per-acre figures showed a significant manurial effect but no manure × year interaction, so that the application of superphosphate was effective early in the trial and remained effective.

There was also a significant year × seed interaction, so that the effect of oversowing grew significantly from year to year.

Sheep Management

Where stock are used as machines to remove herbage growth and to return dung and urine, stocking figures serve only as a rough index of pasture productivity, and should not be interpreted in terms of their carrying capacity. In the present trial, sheep were so used. In addition, the areas were grazed only when ready, thus they were not subjected to differential grazing pressure such as occurs in actual farming practice. There were, however, differences in amount of grazing on each of the treatments which followed a somewhat similar pattern to that of the recorded pasture productivity. Thus the mean annual stocking rate (wethers and dry ewes) on the control areas ranged from $7\frac{1}{2}$ up to 10 per acre, the fertiliser areas from $8\frac{1}{2}$ to $11\frac{1}{4}$ per acre, the seed areas from $7\frac{1}{2}$ up to $10\frac{1}{2}$ per acre, and the fertiliser + seed from 9 to $12\frac{1}{2}$ per acre. There was, in general, an increase in the stock carried on all paddocks over the trial period.

During the first year of the experiment, it was difficult to graze off the hard unpalatable herbage and stock were kept on the paddocks until they were cleaned up. As pastures became increasingly clover dominant, they were more easily controlled by sheep.

The control paddocks were generally more difficult to graze and needed heavier grazing than the improved pastures. The improved paddocks were grazed quickly, while the unpalatable control pastures took longer to clean off at certain times of the year, particularly in summer and autumn when the grasses had run to seed.

Soil Analysis

Superphosphate was applied at the rate of 4 cwt per acre per annum on two of the treatments for the five years of the trial. The plots were grazed to capacity so that those receiving treatment have carried more stock. These factors may have changed the chemical composition of the soil and for this reason composite soil samples from a depth of 0 to 2 in. were taken and analysed by the Soil Bureau.

Table 6 shows that in the control treatment the soil is moderately to strongly acid, moderately leached, low in phosphate, moderate to low in lime, but high in potash. Carbon and nitrogen figures are moderate. The most outstanding results shown by the analyses is that "available" phosphorus has almost trebled in the top 2 inches of the soils receiving superphosphate. There has been no phosphate rise in the clover-alone treatments.

Organic matter and nitrogen have risen where seed or fertiliser has been applied, presumably as a result of greater plant growth, a greater turnover of dung and urine, and the general increase of clover in the pasture. Percentage base saturations also rose in all treatments, the greatest increase being in the fertiliser + seed areas.

From these analyses, it is apparent that additions of superphosphate alone have increased fertility in terms of nitrogen and phosphate. The further increased growth due to fertiliser and seed is probably due to the consistently heavier stocking on these areas. These conclusions are based on a single analysis for each treatment and should be treated with caution.

Conclusion

The trial has demonstrated that this hill pasture can be improved by surface sowing, by topdressing to remedy any mineral deficiency which may be limiting production, and by complete utilisation by stock of all the herbage produced. Large-scale paddock trials on the same area (Suckling 1954) have shown that, with such a combination, it has been possible without the use of supplementary fodder to carry up to $5\frac{1}{2}$ breeding ewes and their lambs per acre. Ultimate production levels are a matter for further study, but it would be safe to suggest that the maximum carrying capacity has not yet been reached.

Acknowledgements

The author wishes to acknowledge the assistance of Messrs. L. E. Madwick, N. Marryott, D. S. Mitchell, A. White, and Miss D. P. Owen, Grasslands Division, Department of Scientific and Industrial Research; Mr. A. C. Glenday, Applied Mathematics Laboratory DSIR; and Mr. M. Young, Soil Bureau, DSIR, for soil analyses.

References

Levy, E. B. 1951: "Grasslands of New Zealand". p.145 Government Printer, Wellington. 344 pp.

Levy, E. B.; Madden, E. A. 1933: The Point Method of Pasture Analysis. *N.Z. J. Agric. 46:* 267-79.

Metson, A. J. 1956: Methods of Chemical Analyses for Soil Survey Samples. *N.Z. Soil Bur. Bull. 12:* 168-75.

N.Z. Soil Bureau 1954: General Survey of the Soils of North Island, New Zealand. *N.Z. Soil Bur. Bull. n.s. 5:* 72-3, 226.

Sears, P. D. 1950: The Establishment of Clovers in Pasture. *Proc. 12th Conf. N.Z. Grassl. Ass.* pp. 132-41.

Suckling, F. E. T. 1951: Results of Recent Experiments on Surface Sowing. *Proc. 13th Conf. N.Z. Grassl. Ass.* pp. 119-27.

————1954: Pasture Management Trials on Unploughable Hill Country at Te Awa. *N.Z. J. Sci. Tech A36(3):* 237-73.

———— —1956: Sheep Management on Improved Hill Pastures. *Proc. N.Z. Soc. Anim. Prod. 16:* 38-47.

PROGRESSIVE REDUCTION OF STORAGE TEMPERATURE FOR APPLES

By C. S. Padfield, Fruit Research Division, Department of Scientific and Industrial Research, Havelock North

(*Received for publication, 30 July 1958*)

Summary

Cool-storage experiments over two seasons with the apple varieties Ballarat, Jonathan, and Sturmer indicate that reducing storage temperature by steps from 38°F to 32°F does not produce more sound fruit than the present recommended practice of holding these varieties continuously at 37–38°F.

Introduction

Some varieties of apples grown in Victoria, Australia, particularly Jonathan, which are susceptible to low-temperature breakdown and deep scald are stored initially at 38°F for six weeks, after which the temperature is reduced by steps to 32°F (Trout *et al.* 1940; Tindale 1957). Under this treatment it is claimed that the incidence of low-temperature disorders is lower and storage life longer than under continuous storage at 37°–38°F. From time to time enquiries have been received as to the value of this treatment for New Zealand-grown varieties which are subject to similar disorders. To evaluate this treatment, experiments were carried out in the two years 1955 and 1956 with the varieties Ballarat, Jonathan, and Sturmer, which are generally stored at 37°–38°F, to avoid low-temperature disorders.

Experimental

Samples of each variety were stored under nine conditions:

A. Continuous storage at 37–38°F.
B. Continuous storage at 34–35°F.
C. Continuous storage at 31–32°F.
D. 28 days at 37–38°F, remainder at 34–35°F.
E. 56 days at 37–38°F, remainder at 34–35°F.
F. 28 days at 37–38°F, 28 days at 34–35°F, remainder at 31–32°F.
G. 56 days at 37–38°F, 56 days at 34–35°F, remainder at 31–32°F.
H. 28 days at 37–38°F, remainder at 31–32°F.
I. 56 days at 37–38°F, remainder at 31–32°F.

Samples consisted of three (Ballarat) or four (Jonathan, Sturmer) bushels.

Each variety was of export maturity, obtained from commercial orchards. Before cool storage all fruit was held for seven days at air temperature, simulating average commercial conditions. On removal fruit was held for fourteen days at 60–65°F before examination.

Percentages of totally and commercially sound apples are presented in Table 1. Commercially sound fruits were those affected so slightly by disorders as to be deemed fit for sale. Detailed results of incidence of individual disorders have been deposited at Fruit Research Division headquarters, Auckland.

TABLE 1. MEAN PERCENTAGES OF TOTALLY AND COMMERCIALLY SOUND APPLES SUBJECTED TO VARYING CONDITIONS OF TEMPERATURE IN COOL STORE

Variety		Treatments									Angles Signif. Diff.		Signif. of F
		A	B	C	D	E	F	G	H	I	5%	1%	
Ballarat 1955	Totally sound												
Picked 19.3.55	Per cent	1	0	0	0	2	1	2	1	1			
Examined after	Equiv. angle				Not	analy sed							
213 days.	Commercially sound												
Size 80	Per cent	5	2	1	8	15	5	15	2	13			
	Equiv. angle	10	6	4	16	23	13	22	7	20	10	14	0.001
Ballarat 1956	Totally sound												
Picked 31.3.56	Per cent	34	27	9	27	39	25	25	14	21			
Examined after	Equiv. angle	36.0	31.4	16.9	31.1	38.4	29.8	29.2	21.4	27.1	6.8	9.4	<0.001
208 days.													
Size 125													
Jonathan 1955	Totally sound												
Picked 14.3.55.	Per cent	22	32	10	25	33	39		21	31			
Examined after	Equiv. angle	27.6	33.7	17.9	29.9	34.8	38.2		26.8	33.6	7.4	10.1	<0.001
126 days.	Commercially sound												
Size 125–138	Per cent	35	49	17	38	49	54		29	48			
	Equiv. angle	36.2	44.0	24.0	39.3	44.4	47.0		32.3	43.5	7.5	10.1	<0.001
Jonathan 1956	Totally sound												
Picked 28.2.56.	Per cent	37	29	16	20	23	19	16	22	16			0.005–
Examined after	Equiv. angle	37.1	32.3	23.8	26.4	28.7	26.1	23.3	27.7	23.2	6.4	8.6	0.001
205 days.	Commercially sound												
Size 150	Per cent	59	48	35	37	41	40	31	44	33			0.005–
	Equiv. angle	50.3	43.7	36.0	36.9	39.2	38.9	34.1	41.9	34.9	6.8	9.2	0.001
Sturmer 1955	Totally sound												
Picked 12.4.55.	Per cent	7	4	8	11	16	22	8	9	24			
Examined after	Equiv. angle	13.0	11.5	14.1	18.5	22.5	26.1	16.8	11.7	28.6			0.10 (NS)
209 days.	Commercially sound												
Size 125–138	Per cent	33	43	23	36	39	47	36	19	54			
	Equiv. angle	34.7	41.1	28.1	36.8	39.1	43.1	36.6	23.5	47.2	11.7	15.8	0.01
Sturmer 1956	Totally sound												
Picked 17.4.55.	Per cent	76	58	14	59	69	47		36	64			
Examined after	Equiv. angle	60.8	49.4	22.0	50.2	55.9	44.4		36.5	53.0	6.8	9.3	<0.001
133 days.	Commercially sound												
Size 113	Per cent	88	77	37	72	87	64		54	78			
	Equiv. angle	69.7	61.5	37.7	58.0	68.9	53.3		47.6	62.3	6.9	9.4	<0.001

DISCUSSION OF RESULTS

Ballarat 1955. On removal, all fruits appeared sound, but during the post-cool-store holding period considerable scald and rots developed. These, together with the large amounts of breakdown and core flush recorded for all treatments, indicated that the holding period had been too long.

Ballarat 1956. The main disorders present were, again, breakdown, core flush, rots, and scald, though these did not develop to the same extent in either degree or severity as in 1955. Breakdown and core flush tended to increase with increased periods at lower temperatures, particularly at 31–32°F, while rots and scald increased at higher temperatures, particularly when the period at 37–38°F was prolonged. Combined losses from rots and scald exceeded those from breakdown and core flush in the best treatments. The reverse was even more marked in the worst treatments. Several treatments were significantly worse, but none were better than standard treatment A.

Jonathan 1955. The reason for discontinuing the experiment at an early date was the high incidence of storage disorders indicated by inspection samples. It will be noted that treatment G could not have been carried through to completion, and was thus not examined.

Incidence of breakdown was high in all treatments, with the majority of fruit severely affected. As expected, the highest percentage (66%) was recorded in treatment C and, surprisingly, the second highest (50%) was found in fruit under treatment A. Least of all (29%) was seen in I. Jonathan spot varied from 2% (H) to 32% (I), but incidence could not be correlated with treatment.

Deep scald occurred under all treatments, though this disorder is not generally expected if the holding temperature is 36°F or higher.

Rots were of both primary and secondary types. The latter predominated as infections on deep-scald lesions. Treatments I, B, E, and F were significantly superior to all others, largely because they suffered less from this combination of deep scald overlaid with rots.

Jonathan 1956. Breakdown increased directly with both time and each successive reduction of temperature. Treatment A had less than 10% of this disorder. Core flush was only serious when Jonathan was held continuously at 32°F (C) and almost non-existent at 38°F continuously (A).

In contrast to 1955, deep scald was negligible.

Incidence of Jonathan spot was high, and could not be correlated with treatment. Except for Jonathan spot, fruit under treatment A was superior to all others.

Summarising the results for both years, it appears that sometimes deep scald can be universal, severe, and unrelated in severity to temperature treatment.

As it is impossible to forecast deep scald, there is no merit in changing the existing recommendation of continuous storage at 37 38°F for the Jonathan apple variety.

Sturmer 1955. This fruit was over-stored by at least a month and this delay is undoubtedly reflected in the high incidence of disorders. Least breakdown (35%) occurred under treatment A, while the most (88%) developed in C.

With rots and superficial scald, increasing periods at the higher temperatures produced increases in both disorders.

Sturmer 1956. High incidence of breakdown caused the curtailment of this experiment before treatment G had been completed. Breakdown ranged from 16% in A to 85% in C, following an almost similar treatment pattern to that occurring in 1955. Once again there were probably no significant differences between treatments in the middle of this range.

Breakdown is the most consistent and serious cause of loss in cool-stored Sturmer apples. Although treatments A and E did not differ significantly in the amount of sound fruit ultimately produced, A developed less breakdown than E and considerably less than I. It would appear that no real advantage is to be gained by altering the present practice of holding this variety continuously at 37–38°F.

Acknowledgement

The assistance of Dr. H. R. Thompson (Applied Mathematics Laboratory) is gratefully acknowledged for statistical analyses.

References

Trout, S. A.; Tindale, G. B.; Huelin, F. E. 1940: *Coun. sci. industr. Res. Aust. Bull. 135.*

Tindale, G. B. 1957: Second Conference of Fruit Storage Investigators, Sydney, 1956. pp. 17-23. CSIRO, Melbourne.

INSECTS AND MITES ASSOCIATED WITH RYEGRASS

By D. C. M. Manson, Horticulture Division, Department of Agriculture, Wellington

(*Received for publication, 22 July 1958*)

Summary

A survey of four ryegrass fields in the Palmerston North district was carried out from October 1956 to January 1957 with the aim of finding what arthropods were present.

Collembola and Acarina were the main groups found, and several species were recorded for the first time.

The general tendency was for populations to increase as the season advanced.

Introduction

Although investigations have been carried out on insect pests of economic importance in pastures of this country, little work has been done in determining the constituents of the fauna of any particular type of pasture. The following survey is a study of the fauna of ryegrass pastures in a North Island area. The main aims are to find what are the main groups of arthropods; to identify the most abundant individuals; and to note any marked changes in the population over a certain period of time.

Method

The locality chosen was that of Palmerston North, as this was most accessible to Wellington. Ryegrass crops, on four separate farms were selected for observation. Three of the fields consisted of perennial ryegrass, while the fourth (A) was short-rotation ryegrass. With the exception of field (D) all the fields were being harvested for seed. Each field of ten acres, was sampled three times during the growing season. The first sampling was carried out in late October 1956, the second sampling 27 November 1956 and the third and final sampling just prior to harvesting for seed. In the case of fields (B) and (C) the final sample was taken on 19 December 1956, while field (A) was sampled on 10 January 1957 and field (D) on 14 January 1957.

Sixteen samples were taken from each field during an examination. The field was traversed in a random fashion, so that the sixteen samples were fairly representative of the ten acres. Each sample consisted of a sod measuring ten inches by six inches which was removed with a sharp spade, as little dirt as possible being included. The sod was enclosed in a plastic bag and samples were later examined in the laboratory. The grass was cut from each sod and spread out over white paper, where insects and mites were readily detected and preserved in alcohol for a later, detailed examination.

Results

The results of this survey are set out in the accompanying table.

The information can be broadly summarised as follows:

In nearly every case, Collembola were the dominant group of insects.

The other main groups were the Acarina, Hemiptera, and Diptera.

There was a tendency for the total number of insects to increase as the season progressed. The exception was field (D). As this was being grazed and not kept for harvesting, this result could possibly be expected.

In the same field fluctuations of individual insects varied markedly from sample to sample. This was most noticeable with the Collembola. Some samples would have more than a hundred of these insects, while others had none.

While total numbers of insects tended to increase from October to January, individual groups within the totals did not always increase. A good example of this is in the Hemiptera (aphids). The number of aphids in fields (A), (B), and (C) reached a peak in November and thereafter declined. The numbers of Diptera in (A) and (B) also declined. The Thysanoptera reached a peak in November and then decreased in numbers in December and January.

Individual species present

(i) Collembola:

The main species of Collembola present in the respective fields were:

A. *Achorutes rossi* Salmon and *Isotomina thermophila* Axelson

B. *A. rossi* Salmon and *Bourletiella arvalis* Fitch

C. *A. rossi* Salmon

D. *A. rossi* Salmon and *Isotomurus palustris* (Mueller)

Most species of Collembola were present in each field. *Achorutes rossi* Salmon was first described in 1941 from a lawn in Wellington, and also off newly dug ground. It is probably fairly widely spread in the southern half of the North Island. *Bourletiella arvalis* Fitch has been recorded (Salmon 1941) from Auckland, Lake Waikaremoana, Lake Wanaka, and Palmerston North. It seems to be generally distributed throughout New Zealand. This is believed to be the first record of the cosmopolitan species *Isotomina thermophila* Axelson and *Isotomurus palustris* (Mueller) in New Zealand.

(ii) Acarina:

The predominant species were the mesostigmatid mites *Parasitus intermedius* Berlese (family Parasitidae) and *Cosmolaelaps* sp. (family Laelaptidae). This is probably the first recording of these two mites in New Zealand. The *Cosmolaelaps* species is thought to be new to science. Six females of *Platyseius mackerrasae* Womersley (family Phytoseiidae) were also discovered. This species was recently described by Mr. H. Womersley (1956) from five females on a tipulid fly taken in Brisbane, Australia in 1951.

Considerable numbers of oribatid mites were also present, but these were more numerous in field (B) than in the others. The commonest were *Platynothrus peltifer* (Koch) (family Camisiidae) and *Zygoribatula* sp. ? *connexa* (Berlese) (family Oribatulidae).

(iii) Hemiptera:

The sole species was the aphid *Rhopalosiphum padi* (L.) Cottier (1953) has recorded this species previously as being found on ryegrass in the Palmerston North area.

(iv) Diptera:

Many larvae and pupae were taken. Approximately 80% of the species consisted of the leaf-mining fly *Cerodontha denticornis* (Panzer), (family Agromyzidae).

TABLE 1. NUMBERS OF INSECTS AND MITES FOUND

Field A		*Field B*		*Field C*		*Field D*	
October 1956							
Collembola	229	Collembola	301	Collembola	159	Collembola	162
Acarina	62	Hemiptera	36	Hemiptera	29	Hemiptera	159
Diptera	17	Acarina	16	Acarina	18	Coleoptera	20
Hemiptera	15	Diptera	5	Coleoptera	15	Thysanoptera	11
Coleoptera	9	Coleoptera	4	Hymenoptera	2	Hymenoptera	8
		Thysanoptera	1	Thysanoptera	1		
				Diptera	1		
	332		363		225		360
November 1956							
Collembola	286	Collembola	965	Collembola	177	Collembola	70
Hemiptera	227	Diptera	101	Hemiptera	84	Acarina	52
Acarina	210	Acarina	99	Diptera	47	Hemiptera	43
Diptera	89	Hemiptera	96	Acarina	38	Coleoptera	26
Thysanoptera	3	Thysanoptera	33	Thysanoptera	10	Hymenoptera	4
		Coleoptera	32	Coleoptera	37	Diptera	14
		Hymenoptera	9	Hymenoptera	2	Thysanoptera	2
	815		1,335		395		211
December–January 1957							
Acarina	566	Collembola	670	Collembola	333	Collembola	112
Collembola	426	Acarina	133	Acarina	102	Acarina	61
Coleoptera	57	Diptera	49	Diptera	74	Thysanoptera	43
Diptera	48	Thysanoptera	24	Coleoptera	27	Diptera	22
Thysanoptera	1	Coleoptera	18	Thysanoptera	8	Coleoptera	11
				Hymenoptera	5	Hymenoptera	8
				Hemiptera	2	Hemiptera	6
	1,098		894		551		363

(v) Coleoptera:

The greatest proportion of the Coleoptera consisted of the Argentine stem weevil, *Hyperodes griseus* Hust. Adults, pupae, and larvae were present. The remainder of the Coleoptera consisted mainly of ground beetles and their larvae (Carabidae) and staphylinid beetles (Staphylinidae).

(vi) Thysanoptera:

Two species were present. They were *Anaphothrips obscurus* (Mueller) and *Anaphothrips secticornis* (Trybom). Both species have been recorded previously in New Zealand. *A. obscurus* (Mueller) was first recorded by Doull (1956) on cocksfoot. *A. secticornis* (Trybom) was recorded by Spiller (1951) on the trunk of poorman orange (*Citrus* sp.) at Auckland in 1940.

Discussion

This survey is of a limited extent and covers only a short period of the year. The method of sampling could no doubt be improved (Johnson *et al.* 1957) but it did have the virtue of being quick and simple to operate.

As may be expected, Collembola and mites were the dominant groups present. A number of species that had not been recorded previously were found in quite large numbers. A more detailed examination of pastures would doubtless result in a number of new species being discovered.

From the economic aspect the most important insects are the Argentine stem weevil *Hyperodes griseus* Hust. and the wheat sheath miner *Cerodontha denticornis* (Panzer).

Morrison (1938) recorded the Argentine stem weevil as causing damage to wheat in Canterbury, as well as to barley and ryegrass. The weevil is widespread through much of New Zealand and can be most destructive to perennial and short-rotation ryegrass. It also damages newly sown pasture and cruciferous crops.

The wheat sheath miner was recorded by Morrison in 1938 as occurring in wheat and barley crops in the mid-Canterbury area. Avice Hamilton (1947) described the habits of this insect.

On the whole the fly appears to be of minor importance, but periodically an increase in numbers may be sufficient to cause damage to ryegrass.

Rhopalosiphum padi (L.) has been recorded (Cottier 1953) as severely infesting young wheat plants and oats, and can be injurious to Gramineae under glass. It appeared to have little effect on the pasture in this survey.

Collembola are usually regarded as comparatively harmless, but seasonal conditions can cause a build up of some species so that they cause damage to plants. *A. rossi* Salmon has been found (Salmon 1943) doing damage in a garden at Karori, Wellington. *B. arvalis*

Fitch has caused damage (Cleland 1955) to crucifers in Ashburton, and it is thought that pasture can be a suitable breeding ground between crop seasons.

ACKNOWLEDGEMENTS

I am greatly indebted to the members of the staff of the Seed Testing Station, Palmerston North for their valuable assistance, as well as to the farmers of that district who allowed me to examine their ryegrass fields. For identification of many of the insects and mites, thanks are due to Dr. J. T. Salmon of Victoria University College, Wellington, Mr. H. Womersley of the South Australia Museum, Adelaide, and Dr. W. J. Hall of the British Museum, London. Mr. L. Morrison and Mr. K. M. Doull gave helpful criticism.

REFERENCES

Cleland, J. W. 1955: Distribution and Control of Springtails in Cruciferous Crops and Pastures. *N.Z. J. Agric. 91:* 13.

Cottier, W. 1953: Aphids of New Zealand. *N.Z. Dep. sci. industr. Res. Bull. 106:* 155-9.

Doull, K. M. 1956: Thrips Infesting Cocksfoot in New Zealand. I. The Thrips Fauna of a Cocksfoot Seed Crop. *N.Z. J. Sci. Tech. A38:* 52-5.

Hamilton, Avice 1947: Ryegrass Attacked by Wheat Sheath Miner. *N.Z. J. Agric. 75:* 447.

Johnson, C. G.; Southwood, T. R. E.; Entwistle, H. M. 1957: A New Method of Extracting Arthropods and Molluscs from Grassland Herbage with a Suction Apparatus. *Bull. Ent. Res. 48 (1):* 211-18.

Morrison, L. 1938: Surveys of the Insect Pests of Wheat Crops in Canterbury and North Otago during the Summers of 1936-37 1937-38. *N.Z. J. Sci. Tech. A20:* 142-55.

Salmon, J. T. 1941: The Collembola Fauna of New Zealand. *Trans. roy. Soc. N.Z. 70:* 282-431.

——— ———1943: New Records of Collembola from New Zealand. *Ibid. 72:* 373-88.

Spiller, D. 1951: Notes on Thysanoptera. *N.Z. J. Sci. Tech. B33:* 142-3.

Womersley, H. 1956: On Some New Acarina—Mesostigmata from Australia, New Zealand, and New Guinea. *J. Linn. Soc. (Zool.) 42:* 505-99.

TWO DISEASES OF PLUM CAUSING DISTORTION AND INTERNAL NECROSIS OF FRUITS

By E. E. Chamberlain[1], J. D. Atkinson[2], and J. A. Hunter[1]

I. PLUM FRUIT CRINKLE, A VIRUS DISEASE RESEMBLING PLUM POX OF EUROPE

(*Received for publication, 1 September 1958*)

Summary

Plum fruit crinkle, a virus disease of plums, has been found in New Zealand on three trees of the Japanese plum variety Sultan.

It causes crinkling and internal necrosis of fruits and transient mottling and faint chlorotic line patterns on leaves. In many respects symptoms resemble those of the serious virus disease known as plum pox in Europe.

It is possible that plum pox and plum fruit crinkle are caused by the same disease and that variations in symptoms are the result of host, virus strain, or climatic differences.

Plum fruit crinkle has been transmitted experimentally from Sultan to Sultan, George Wilson, and Shiro plum trees.

An attempt is being made to eradicate the disease from New Zealand.

Introduction

Plum pox, a serious disease of plums, has been known in Bulgaria and Yugoslavia for many years. It was described as a new virus by Atanasoff (1932) who stated that it was then widely spread in south Bulgaria. Christoff (1938) estimated that the number of infected trees in Bulgaria exceeded one million.

In a recent survey of the plum pox position in Yugoslavia, Yossifovitch (1956) stated that it was first noticed in that country in 1936 and that by 1956 incidence was estimated at more than eleven million trees.

The disease, which ruins the fruit, is spread by vegetative propagation and by the aphid *Bachycaudus helichrysi* (Kaltenbach) (Atanasoff 1934).

There is little doubt that in early accounts of the disease European workers attributed to plum pox symptoms which were caused by other stone fruit viruses. Atanasoff (1932) and Christoff (1938) suggested that several virus diseases reported from non-European countries might be caused by the plum pox virus. The identity of such diseases with plum pox has not been confirmed and, as far as we can ascertain, there is no record of the disease outside Europe. Within Europe, however, it is widespread, having been recorded from Bulgaria, Czechoslovakia (Atanasoff 1932), Yugoslavia (Christoff 1947), Hungary (Husz and

[1]Plant Diseases Division,
[2]Fruit Research Division, Department of Scientific and Industrial Research

Klement 1950) England (Harris 1956), Greece (Yossifovitch 1956), and Germany (Schuch 1957).

In January 1951 specimens of Sultan plum fruits showing symptoms somewhat similar to those of plum pox were received at this laboratory from a Hawke's Bay orchard. It has since been shown that the symptoms were caused by a transmissible virus. Although the New Zealand disease has a number of features in common with European plum pox, there appear to be certain differences, and the fact that the former disease occurs on Japanese plum (*Prunus salicina*), and the latter on European plum (*Prunus domestica*), makes strict comparison impossible. In the meantime the two diseases are being regarded as different and the name plum fruit crinkle has been given to the New Zealand disease.

SYMPTOMS

Plum Fruit Crinkle

On infected Sultan plum trees, symptoms first appear on fruits

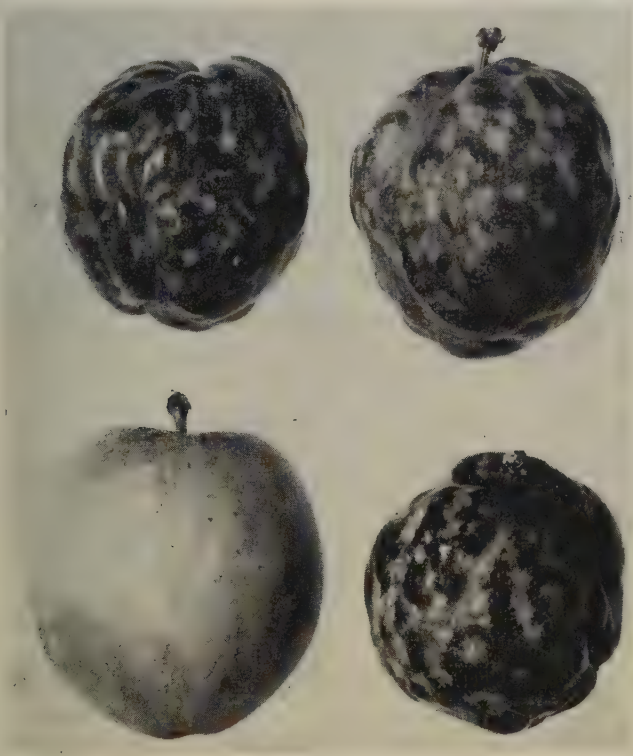


Photo: J. W. Endt

Fig. 1.—Plum fruit crinkle: Sultan plums showing light-coloured sunken areas producing a wrinkled appearance. Healthy fruit at lower left. The deeper colour of infected fruits indicates the degree of premature ripening caused by the disease. × 1.

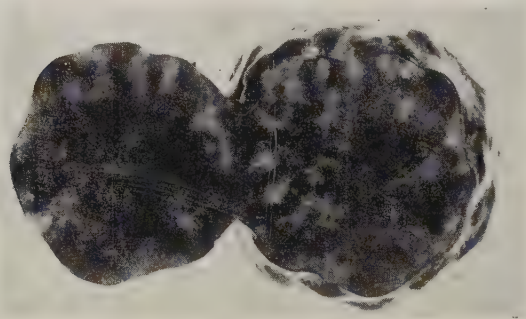


Photo: S. A. Rumsey

Fig. 2.—Plum fruit crinkle: Sultan plum showing light-coloured necrotic areas in flesh. × 1.

Photo: S. A. Rumsey

Fig. 3.—Faint chlorotic line pattern on leaf of Sultan plum infected with plum fruit crinkle. × ¾.

nearing maturity as numerous light-coloured spots or ring spots which become depressed to give fruits a wrinkled appearance (Fig. 1). The fruits begin to colour 10–14 days earlier than those on healthy trees and tend to drop prematurely. Scattered through the flesh are light-brown, necrotic, spongy areas (Fig. 2), which frequently penetrate to the stone. The stone itself is normal. Fruits are dry, lack sweetness, have an unpleasant flavour, and are commercially valueless. Transient light-coloured areas appear on leaves near the base of current-year's shoots, while faint chlorotic line patterns (Fig. 3) occur on some later-formed leaves. In the nursery, infected trees make normal growth, but infected orchard trees appear to lack vigour and are smaller than healthy trees of the same age.

Symptoms have also been observed on experimentally infected George Wilson and Shiro plum trees. On these varieties the disease may not have given full symptom expression as observations were made in the season following inoculation when fruit symptoms had appeared on only portion of the crop. At this stage, foliage symptoms had not appeared on either variety.

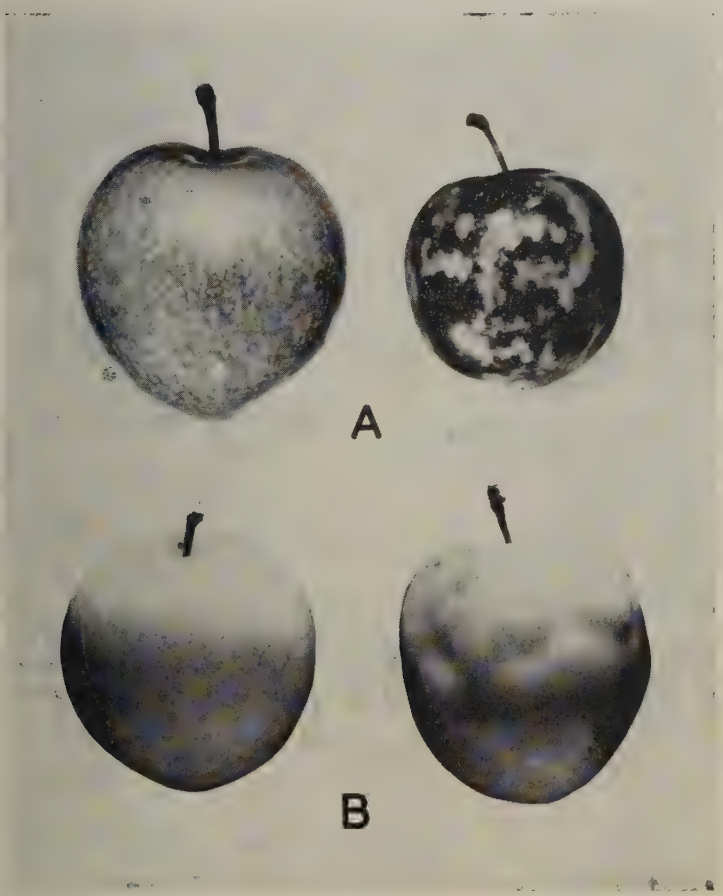


Photo: S. A. Rumsey

Fig. 4.—Plum fruit crinkle: Symptoms on fruits. A. George Wilson: normal fruit on left. Light-coloured areas are conspicuous but only slightly depressed. B. Shiro: normal fruit on left × 1.

On fruits of George Wilson, light-coloured areas were very conspicuous (Fig. 4A), but they were only slightly depressed. Reddish-brown, necrotic areas were present in the flesh but occurred mainly near the surface and only occasionally penetrated to the stone. Effect on fruit quality was less than with Sultan, juice content being only slightly reduced and flavour little affected. Maturity was advanced by a few days.

In the yellow-fruited variety Shiro, fruits showed slight distortion without change in colour (Fig. 4B). Areas of light-brown, slightly spongy tissue were present in the flesh but there seemed to be little change in juice content, flavour, or maturity.

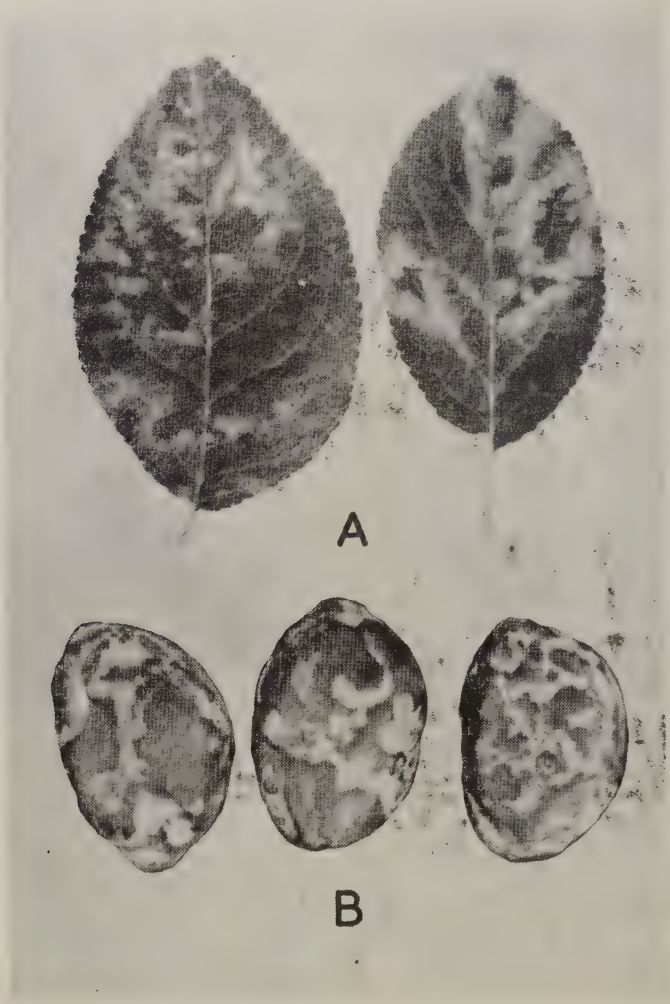


Fig. 5.—Plum pox of Europe. Symptoms on the European variety Pozegaca. A. Leaves. B. Fruits.
After Yossifovitch.

Plum Pox

The symptoms described below have been taken from descriptions made by Atanasoff (1932), Christoff (1938), and Yossifovitch (1956) and apply mainly to the European plum variety known as Kustendil in Bulgaria and Pozegaca in Yugoslavia.

Symptoms on leaves consist of indistinctly outlined chlorotic spots, ring spots and broad line patterns (Fig. 5A). Infected trees make normal growth in their early years but later, weak development of summer shoots

is apparent. No symptoms appear on fruits until they reach full size when they begin to ripen 10–15 days earlier than those on healthy trees. About this time fruits develop one or more blue spots or ring spots. Gradually the spots become sunken, the surface taking on a pock-marked or wrinkled appearance (Fig. 5B). Tissues underlying the spots are usually necrotic, rust coloured, dry and shrunken, lesions often extending to the stones on which dark ring spots develop. The fruits have a low sugar content and a taste so unpleasant that they are uneatable. Symptom expression varies from season to season and in some years infected trees may produce a crop of normal fruits. Sometimes the disease remains localised for a long time in one part of a tree.

INCIDENCE OF PLUM FRUIT CRINKLE

Plum fruit crinkle has been found on one Sultan plum tree in a small block of seven-year-old trees in the Hawke's Bay district and on two 22-year-old Sultan trees in a block of 21 trees in an Auckland orchard.

TRANSMISSION EXPERIMENTS

In a trial commenced in the 1950–51 season, five cherry-plum stocks were worked with buds from an infected Sultan tree. Scions produced their first crop in the 1952–53 season, all fruits showing typical symptoms. In that season three of the five trees were worked with buds from a healthy Sultan tree. Each season since then, fruits on the originally infected scions have shown symptoms of plum fruit crinkle. The originally healthy scions all fruited in the 1956 57 season and each of the 24 fruits carried on these scions showed typical symptoms. Three healthy Sultan check trees carried a crop each year from 1952–53 and in no season did any symptoms appear.

In the autumn of 1956, buds from an infected Sultan tree were worked to two young George Wilson trees and one young Shiro tree. Both George Wilson trees bore a small crop during the 1956–57 season. On one tree, nine George Wilson fruits out of eleven showed symptoms, and, on the other, five out of nine fruits developed symptoms. An adjacent George Wilson check tree carried 21 normal fruits. The Shiro tree bore a crop of 15 fruits, five of which showed symptoms. No symptoms developed on nine fruits on two adjacent Shiro check trees.

In another transmission trial extending over four years including two fruiting seasons, neither foliage nor fruit symptoms developed on the Satsuma variety. It would appear that this Japanese plum is either highly resistant or a symptomless carrier of the disease.

Leaves of cherry plum (*Prunus cerasifera*) stock shoots growing from the base of infected Sultan trees also failed to develop symptoms.

ERADICATION

Although plums are not a major crop in New Zealand there are over 110,000 trees in commercial orchards and large numbers are grown in domestic gardens. They are of considerable importance for jam making and bottling, and as a fresh fruit.

Because of its serious effect on fruit and its ready transmission by propagation methods, plum fruit crinkle is regarded as a dangerous disease and an attempt is being made to eradicate it from New Zealand. All known naturally infected trees and most of the experimental material has been destroyed. Some infected material is being maintained under quarantine conditions for further experimental work.

Discussion

Plum fruit crinkle and plum pox are similar in many respects but a comparison of symptoms shows that there are certain differences. In particular no gum-filled pockets in the flesh or dark markings on stones have been observed with the New Zealand disease. Also foliage symptoms are much less conspicuous. However, Christoff (1947) recorded considerable variation in fruit and foliage symptoms of plum pox on different varieties of European plum.

It is possible that the European and New Zealand diseases are caused by the same virus and that variation in symptoms is the result of differences in host, virus strain, or climate. Further work is required to determine whether the two diseases are caused by the same or different viruses.

II. PLUM FALSE CRINKLE, A PHYSIOLOGICAL DISORDER OF PLUMS

Summary

A physiological disorder causing malformation and internal necrosis of fruits has been found on the three Japanese plum varieties Billington, George Wilson, and Wright's Purple.

Only on the variety Billington are fruit symptoms accompanied by foliage symptoms.

The disorder occurs in both the Auckland and Hawke's Bay districts.

Symptoms appear to differ from those of physiological diseases causing malformation and internal necrosis of plum fruits in other countries.

The name plum false crinkle has been given to this disease.

Introduction

Surveys to determine the incidence of plum fruit crinkle led to the discovery of distortion and internal necrosis on fruits of the Japanese plum varieties Billington, George Wilson, and Wright's Purple. Although symptoms are somewhat similar to those of plum fruit crinkle all attempts to reproduce or transmit the disease have failed so it must be regarded as a physiological disorder.

Physiological disorders recorded in other countries as causing distortion and internal necrosis of plum fruits appear to differ from the condition occurring in the Dominion.

Heat spot, also known as Kelsey spot, recorded from South Africa (Dippenaar 1932, 1935, 1937) and U.S.A. (Proebsting 1936) differs

from the New Zealand disease in having darker, more clearly defined internal lesions which often develop cavities. It develops following periods of excessively high temperature which do not normally occur here. Hard flesh spots described in U.S.A. by Adams (1923), and crinkle of Japanese plums described by Carne (1926) in Western Australia, appear to resemble heat spot.

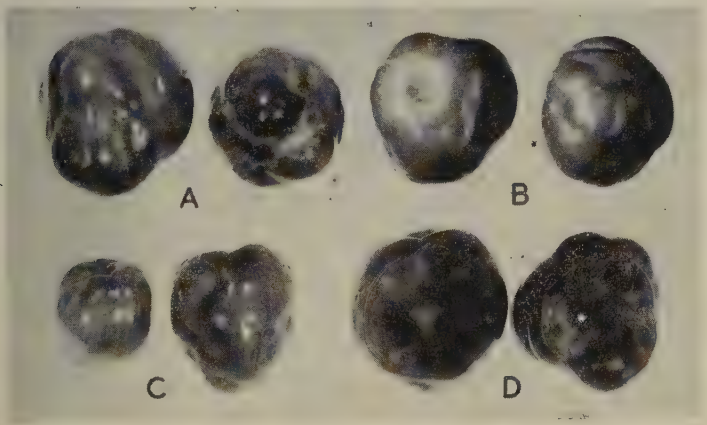
Drought spot or gum spot which has been recorded from Canada (McLarty 1925) and South Africa (Dippenaar 1932, 1935) causes small deep-seated brown spots overlying convex cavities, whereas the New Zealand disorder frequently has no surface spots and the internal lesions are diffuse and inclined to be spongy. The New Zealand disease does not appear to be associated with drought conditions.

Boron deficiency recorded on European plum by Hansen and Proebsting (1949) in U.S.A. and by Bollard (1955) in New Zealand causes a wrinkling of fruit quite unlike symptoms of the disorder under consideration.

Symptoms occurring on the three plum varieties in New Zealand may have more than one cause, but until further information is available, all have been grouped under the name plum false crinkle.

SYMPTOMS

The most characteristic symptoms of plum false crinkle are: bold crinkling and distortion of fruits (Fig. 6A, B, C), which develop while fruits are still immature, and occurrence of light, rust-coloured, necrotic areas in the flesh (Fig. 6D). There is no marked change in fruit colour although light-coloured areas may occur in depressions on severely distorted Billington and George Wilson plums. In the varieties Billington and Wright's Purple the whole crop may be affected and



Photos A, C, and D: S. A. Rumsey, B: H. Powell.

Fig. 6.—Plum false crinkle. Symptoms on fruits. A. Billington, B. George Wilson. C. Wright's Purple. D. Internal necrotic areas on Billington plums. × ⅗.

symptoms may recur each season, but, in George Wilson, symptoms occur on comparatively few fruits per tree and do not recur each season. Flavour of fruit is not affected. No foliage symptoms have been observed on George Wilson or Wright's Purple, but, on Billington, leaves are smaller than normal and show a faint light- and dark-green mottling, while growth of annual shoots is retarded.

The above symptoms differ from those of plum fruit crinkle in the boldness of crinkling, development of distortion on immature fruits, and failure of affected fruits to develop an unpleasant flavour.

Incidence

Plum false crinkle has been found on a single Billington plum tree in Hawke's Bay and on several trees in a block of Wright's Purple near Auckland. In the 1955–56 season symptoms were found on a small number of fruits on 11 trees in a block of 15 George Wilson trees in an Auckland orchard. In Hawke's Bay in the same season, the condition was general in a block of 350 young George Wilson trees and occurred on a few fruits in a block of older trees.

Transmission Experiments

With all three varieties attempts were made to transmit the disease to healthy trees of the same variety. In no case was the disease reproduced on scions from affected trees or transmitted to healthy scions.

Acknowledgements

The authors gratefully acknowledge the assistance given by members of the Horticulture Division, Department of Agriculture, who discovered both diseases in the field, provided information on field symptoms, forwarded specimens and propagation material, carried out orchard surveys to determine their incidence, kept close watch for field spread, and, where necessary, arranged destruction of trees.

References

Adams, J. F. 1923: *Pl. Dis. Bull. Suppl. 28:* 268-392. (Abstr. in *Rev. appl. Mycol. 3:* 337-8.)

Atanasoff, D. 1932: *Yearbook Univ. of Sofia Fac. of Agric. Sofia 11:* 49-70. (Abstr. in *Rev. appl. Mycol. 12:* 229-30).

———1934: *Annu. Univ. Sofia. 13:* 9-42. (Abstr. in *Rev. appl. Mycol. 14:* 367-8.)

Bollard, E. G. 1955: *N.Z. Dep. sci. industr. Res. Bull. 115,* 52 pp.

Carne, W. M. 1926: *J. Dep. Agric. W. Aust., 2nd Ser., 3:* 175. (Abstr. in *Rev. appl. Mycol. 5:* 676-7.)

Christoff, A. 1938: *Phytopath. Z. 11:* 360-422.

——— 1947: *Bull. Chambre Cult. nat. Sofia. Ser. Biol. etc. 1:* 261-96. (Abstr. in *Rev. appl. Mycol. 27:* 27.)

Dippenaar, B. J. 1932: *Fmg. in S. Afr. 6:* 525-6. (Abstr. in *Rev. appl. Mycol. 11:* 521-2.)

———————1935: *Ibid. 10:* 333-6.

———————1937: *Ibid. 12:* 83-5.

Hansen, C. J.; Proebsting, E. L. 1949: *Proc. Amer. Soc. hort. Sci. 53:* 13-20.

Harris, R. V. 1956: *Rep. E. Malling Res. Sta. 1955,* pp. 37-43.

Husz, B.; Klement, Z. 1950: *Agrartud. egy. Evkon. I* (*Agrartud. egy. 14*), 1, 83-94. (Abstr. in *Rev. appl. Mycol. 36:* 252.)

McLarty, H. R. 1925: *Rep. Dep. Agric. Can. 1924.* pp. 72-8. (Abstr. in *Rev. appl. Mycol. 5:* 536-7.)

Proebsting, E. L. 1936: *Proc. Amer. Soc. hort. Sci. 34:* 272-4.

Schuch, K. 1957: *Mitt. biol. ZentAnst. Berl. 88,* 96 pp.

Yossifovitch, M. 1956: *Tijdschr. Plziekt. 62:* 56-9.

SOIL-MOISTURE AND SOIL-TEMPERATURE STUDIES UNDER RADIATA PINE, WHAKAREWAREWA FOREST, 1954-55

By G. M. Will, New Zealand Forest Service, Whakarewarewa

(*Received for publication, 5 August 1958*)

Summary

Soil-temperature and soil-moisture changes during the year October 1954–October 1955 have been studied in two experimental plots in Whakarewarewa Forest. One plot was in a 39-year-old stand of radiata pine and the other in a seven-year-old stand of the same species. Changes down to a depth of 9 ft 9 in. have been measured and correlated with the rainfall and mean daily temperatures.

Differences between plots were found and their relationships to variations in the canopy, to thickness of litter, and to air movement in the stand are discussed.

Both gypsum and fibreglass soil-moisture units were used, but the latter proved more satisfactory because of a more rapid response to a lowering of the soil-moisture content.

Introduction

Approximately 500,000 acres of radiata pine (*Pinus radiata* D. Don) and other exotic conifer species have been planted on the central volcanic plateau of North Island, New Zealand. The greater part of the planted area lies between 1,000 and 2,000 ft above sea level, between latitudes 38° and 39° S. and receives a well distributed rainfall of 50–60 in. per annum. The mean annual temperature is 50°F and unseasonal frosts are a feature, particularly on the upland plateau.

The central region has a long history of volcanic activity and almost all soils are derived from volcanic-ash beds, which mantle the landscape. The main soil-forming ash beds described by Grange (1929, 1931) are the Taupo ash (pumice) which forms the surface soil over 800,000 sq. miles, the Kaharoa ash, and the Tarawera lapilli and Rotomahana "mud" erupted in 1886. Soils derived from Taupo and Kaharoa ash beds are classified as yellow-brown pumice soils, and those from the 1886 ejecta are recent soils from volcanic ash (N.Z. Soil Bureau 1954).

Most of the exotic forest is sited on very friable and well drained pumice soils, and lateral roots are concentrated mainly in the top 12 in. of soil. Where these soil layers overlie 18 in. or more of loose pumice gravel beds or compact ash layers, few sinker roots penetrate through into the fossil soils below. After summers with infrequent falls of rain (particularly the summer of 1945–46) many trees have failed to recover from damage caused by the wood wasp (*Sirex noctilio* Fabr.) and it has been suggested that physiological drought within the trees has lowered its resistance to the attacks of the symbiotic fungus carried by *Sirex*.

In the early spring of 1954, soil-moisture-recording instruments were installed in Whakarewarewa State Forest, near Rotorua, with the

object of studying soil-moisture and soil-temperature changes in general and of developing techniques to be applied later in studies of coarse pumice soils more typical of the region.

To enable a comparison to be made, two types of moisture unit were buried in the soil; the gypsum type as first described by Bouyoucos and Mick (1940) and the fibreglass unit developed by Colman and Hendrix (1949). The latter contains a thermistor for temperature measurement.

SITE OF INVESTIGATIONS

The sites chosen for installing the soil-moisture units were two one-acre experimental plots in Whakarewarewa Forest, Rotorua Conservancy. Both were well inside uniform stands of trees, so results obtained should be representative of conditions found in the two types of stand. Plot R.207 was in a stand of radiata pine which regenerated after clear felling in 1947. In 1954 the stocking was 1,500 trees per acre with an average height of 35 ft; the canopy was almost completely closed. Plot R.209/1 was in an unthinned stand of radiata pine planted in 1915 at 8 × 4 ft spacing. Mortality had reduced the stocking to 100 trees per acre by 1954. The average height of the stand was then 116 ft, with crown cover about 50%.

Plot R.207 was at an altitude of 1,800 ft on a southerly slope of 12°; the soil type is Rotomahana shallow sandy loam. Plot R.209/1 was on a slope of 30° at 1,950 ft on Rotomahana shallow sandy loam, hill soil (N.Z. Soil Bureau 1954). Despite the difference in slope both profiles show approximately 6 in. of Rotomahana "mud" deposited in 1886 overlying 3 in. of old humus soil derived from Taupo ash, which in turn overlies fossil soils derived from the Mamaku and Rotorua ash showers (Grange 1931). Fig. 1 shows a diagrammatic representation of each profile and the positions at which moisture units were placed.

The depth of fresh and decomposing litter in plot R.207 was $1\frac{1}{2}$–2 in. while in plot R.209/1 the depth was $\frac{1}{2}$–1 in.

In both plots the main rooting zone was at a depth of 6–18 in. in the lower part of the Rotomahana "mud" and in the old Taupo ash topsoil.

DESCRIPTION OF INSTRUMENTS, THEIR CALIBRATION AND INSTALLATION

Description

The gypsum soil-moisture units and resistance meter used have been described by Aitchison *et al.* (1951) and Aitchison (1953). The fibreglass units are of the type described by Colman (1950). The Aitchison (1953) resistance meter was used to make all resistance readings of soil-moisture units and thermistors.

Calibration

The methods of calibration of soil-moisture units were similar to those employed by Aitchison *et al.* (1951). After the units had been embedded in about 500 g of soil placed in a 2-litre glass beaker, sunflower plants were grown in the soil. Waterproof paper covers, around the stems of the plants and tied over the tops of the beakers, confined

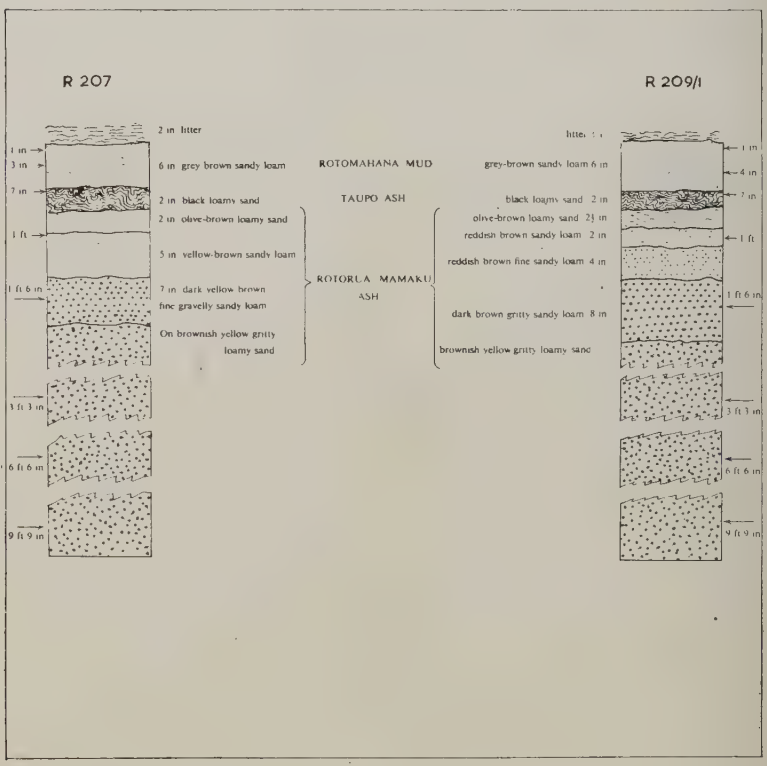

R 207
R 209/1
2 in litter
6 in grey brown sandy loam
ROTOMAHANA MUD
2 in black loamy sand
TAUPO ASH
2 in olive-brown loamy sand
5 in yellow-brown sandy loam
7 in dark yellow brown fine gravelly sandy loam
ROTORUA MAMAKU ASH
On brownish yellow gritty loamy sand
grey-brown sandy loam 6 in
black loamy sand 2 in
olive-brown loamy sand 2½ in
reddish brown sandy loam 2 in
reddish brown fine sandy loam 4 in
dark brown gritty sandy loam 8 in
brownish yellow gritty loamy sand

Fig. 1.—Soil profiles showing the different layers and the depths at which moisture units were placed (indicated by arrows).

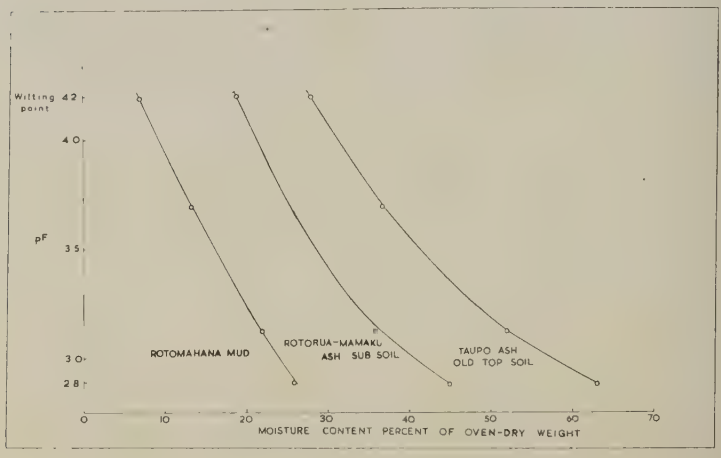


Fig. 2.—Moisture content/pF curves for three soil types.

moisture losses to the transpiration from the plants. Resistance readings were taken during several drying cycles to give resistance-moisture content calibration curves for each moisture unit.

The gypsum units were from a batch calibrated by the Commonwealth Scientific and Industrial Research Organisation, Adelaide, Australia, for resistance against pF in a pressure-membrane apparatus. Using these units as standards and also the observed field capacities and wilting points, resistance-pF calibration curves were obtained for the fibreglass units.

The water-holding capacity of the soil, in terms of available moisture, varies with layers in the profile (see Fig. 2); Rotomahana "mud" has a capacity of 15% of its dry weight, the old topsoil 25%, and the subsoil 17%. The three layers also have different wilting points: Rotomahana "mud" at 7% moisture content, the old topsoil 28%, and the subsoil 19%. Because of these differences it was thought better to use pF rather than moisture content as a measure of the moisture status of the soil when reporting the results of field studies.

The moisture units were calibrated in three groups:

1. In soil from 0–6 in.—grey-brown fine sandy loam - Rotomahana mud.
2. In soil from 8–10in.—a black loamy sand - - - - Taupo ash
3. In soil from 12–18 in.—a yellow-brown sandy loam - Mamaku ash

Within each group there were equal numbers of gypsum and fibreglass units to enable them to be installed in the field in pairs for comparative purposes.

Installation

In each experimental plot, pairs of units, one gypsum and one fibreglass, were placed at various depths in the layer of soil in which they had been calibrated. Those within 6" in. of the surface of the mineral soil were placed individually, so as to distrub the soil as little as possible. Owing to the loose nature of the soil it was necessary to dig pits to install moisture units at greater depths. Pits 10 ft deep were dug and the units installed at depths down to 9 ft 9 in. by tunnelling several inches horizontally from the uphill face of the pit, embedding the units and repacking the soil in the tunnels. This was done so that no unit was vertically beneath another, and all the electrical leads were given a downward slope for several inches before they were brought to the surface. The pits were carefully refilled to give as nearly natural conditions as possible.

RESULTS

From the results obtained during the laboratory calibrations and the log resistance – pF calibration curve for the gypsum units supplied by CSIRO, it has been possible to draw moisture content - pF curves for the three layers of soil found in the area of the experimental plots. These curves are shown in Fig. 2.

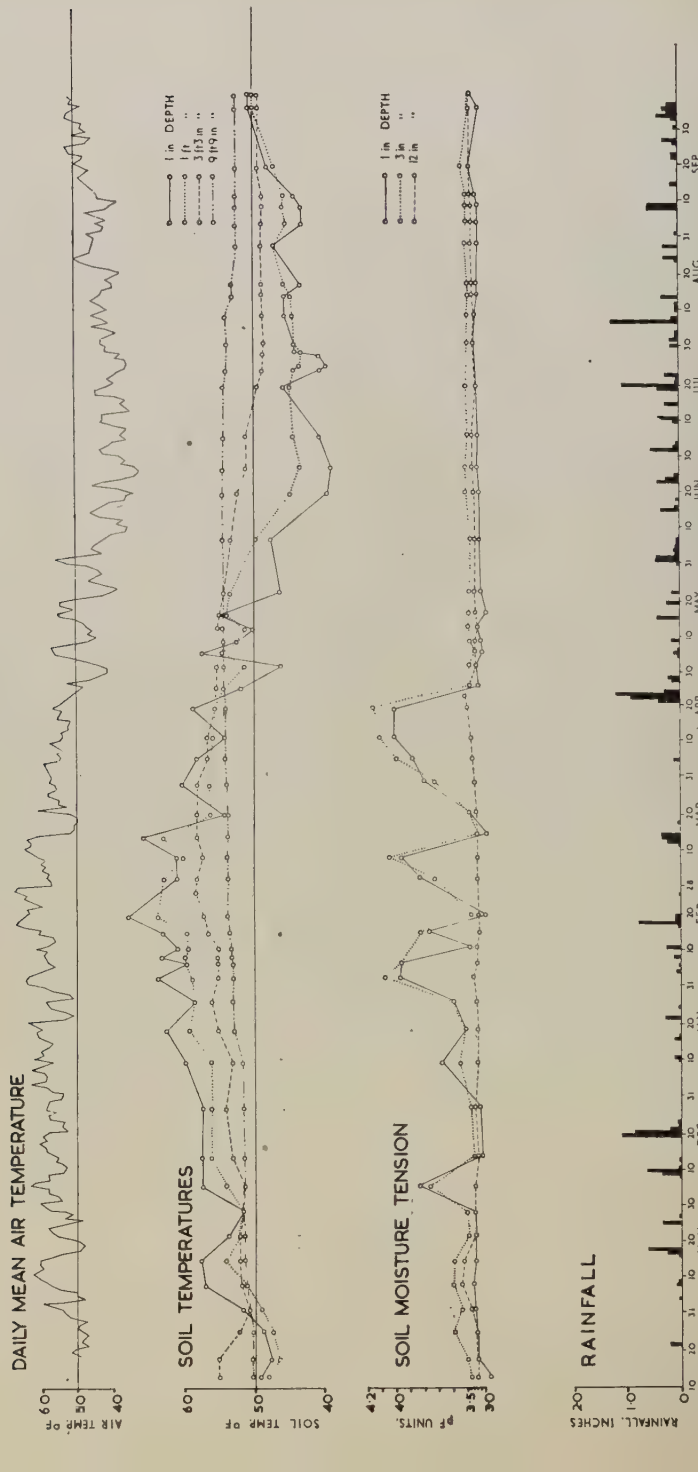


Fig. 3.—Rainfall, moisture, and temperature records for one year beneath 7-year-old *P. radiata* regeneration (Plot R.207).

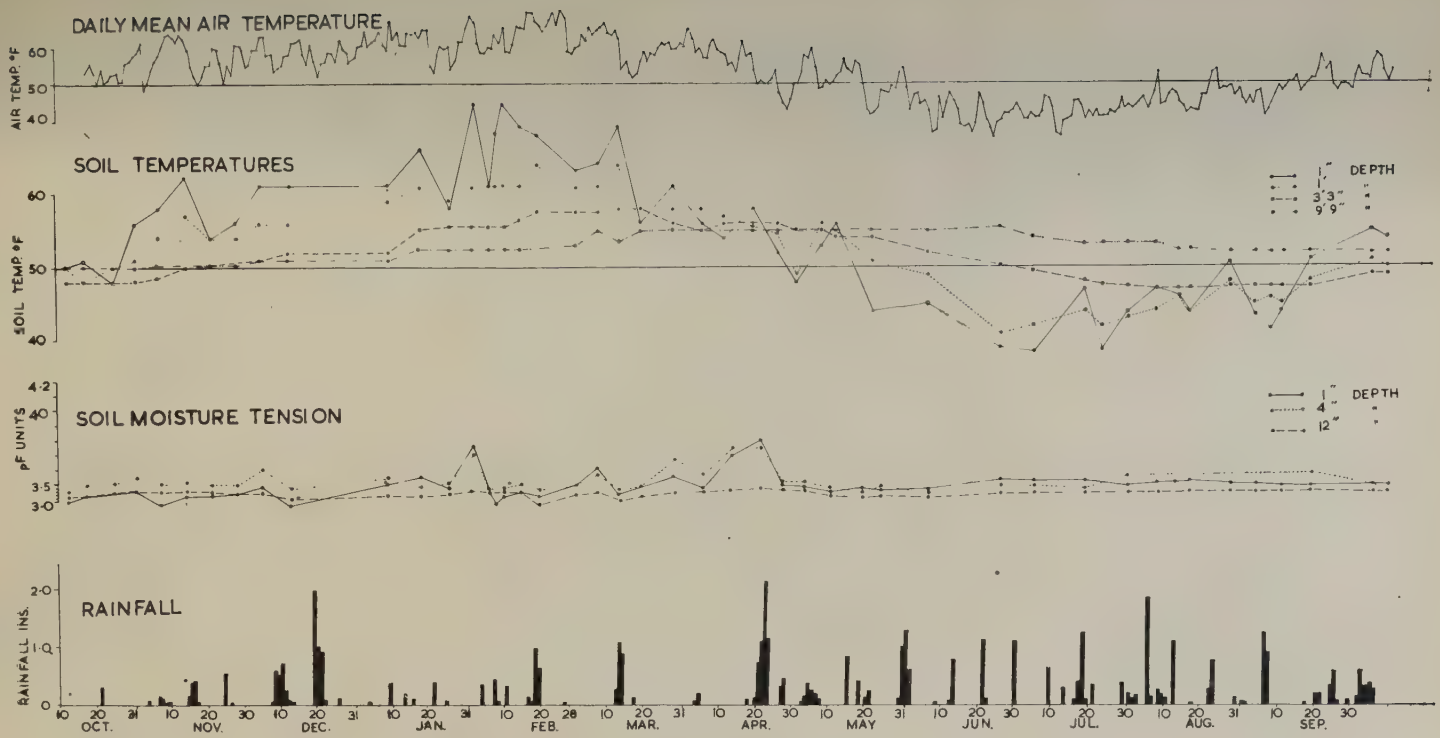


Fig. 4.—Rainfall, moisture, and temperature records for one year beneath 39-year-old *P. radiata* regeneration (Plot R 209/1).

The results of the field experiments are given in Fig. 3 and 4. Rainfall values are plotted beneath the graphs of changes in pF for comparison. These values were obtained from master rain gauges placed, one in each plot, in the immediate vicinity of the area where the moisture units were buried. The year's rainfall as recorded in the master gauge in plot R.207 was 24.7 in. while the average rainfall over the whole plot, derived from randomly placed gauges,* was 35.6 in. The figures for plot R.209/1 were 48.0 and 38.6 in. respectively. Because of the position of the master gauge, the effective rainfall for each area in which moisture readings were taken would lie between the value for the master gauge and the average over the plot. The rainfall at a control site in the open between the two plots was 59.4 in.

Air temperatures are mean daily temperatures recorded in a climatological screen installed in each plot close to the position of the moisture units and thermistors, and 4 ft above the ground. These climatic data are derived from an associated study by this institute of microclimatic variations within a number of stands of radiata pine, fuller details of which are to be published at a later date.

The graphs in Fig. 3 and 4 have been constructed using the results from the fibreglass units alone. This has been done because the fibreglass units were found to be more sensitive than the gypsum units to moisture changes at low pF. There were times when rain brought the soil back to field capacity after a short period of dry weather. During such periods the resistance of the gypsum units remained constant throughout, but the fibreglass units did show some increase in resistance before the soil returned to field capacity.

Units were placed at depths of 1 in., 3 in., or 4 in., 7 in., 1 ft, 1 ft 6 in., 3 ft 3 in., 6 ft 6 in., and 9 ft 9 in. below the surface of the mineral soil but not all the results have been used in Fig. 3 and 4. Some have been omitted to avoid confusion through having too many lines plotted on the one graph. The soil-temperature variations at the depths not graphed were between those shown. The same is true of the soil-moisture results, which are reported only to a depth of 12 in.; at greater depths no change in moisture content was measured at any time during the year.

Discussion

The pF graphs show that the Rotomahana "mud" topsoil dried out appreciably during periods of dry weather, but after a fall of rain greater than 1½ in. (as measured in the open) it returned within a short time to field capacity, or nearly so. Smaller amounts of rain caused some lowering of the pF values, but it must be remembered in such cases that a large proportion of the rainfall is caught up in the foliage of the trees and by the litter, allowing the rain to evaporate without reaching the mineral soil. The thicker layer of litter together with the denser canopy will have played its part in preventing a greater proportion of the rainfall in plot R.207 from reaching the ground. This is reflected in the generally higher soil-moisture tensions in the surface layers of this plot during the summer.

* These gauges were moved to randomly selected sites after each rainstorm,

Nothing was observed to suggest that the trees in plot R.207 were affected by the drying out of the topsoil to wilting point (pF 4.2) at times during the late summer. As most of the fine feeding roots lie below this zone at a depth of 6–18 in., this is perhaps not unexpected. But it is of interest that in this main rooting zone at a depth of 12 in. it was only after prolonged periods of dry weather that any appreciable rise in pF value occurred.

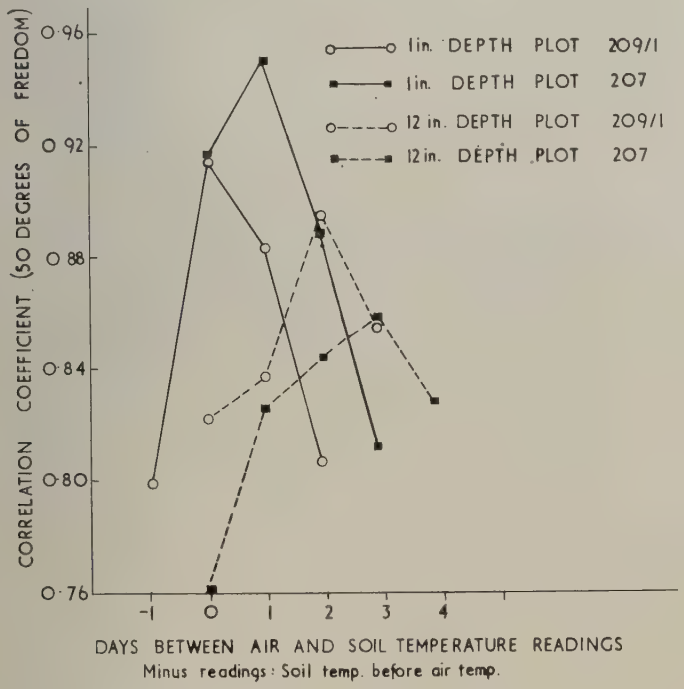


Fig. 5.—Correlation coefficients between soil temperature and mean air temperature at various time intervals.

In Fig. 5 the correlation coefficients between soil temperature (measured at about 11 a.m.) and mean air temperature for the same day, one day before, two days before, etc., are shown. This has been done for two depths, 1 in. and 12 in. In plot R.209/1 the temperature of the soil at 1 in. shows a peak correlation with the same day's air temperature, while at a depth of 12 in. the maximum correlation is with the air temperature of two days before. In plot R.207 the peak correlations are with the air temperature of the previous day and three days before, respectively. This greater delay in responding to changes in air temperature can probably be attributed to a thicker litter layer and a much lower rate of air movement through the stand. Total air movement during the year in plot R.207, recorded by anemometers 4 ft above the ground, was only about 3% of that in plot R.209/1.

At 9 ft 9 in., the small temperature changes that did occur lagged several months behind changes at the surface.

At a depth of 1 in., slightly greater seasonal variations in temperature were recorded beneath the older stand of trees (plot R.209/1)—38° to 72°F, compared with 39° to 68°F in plot R.207. This was to be expected since the more open nature of the stand allowed some direct radiation to reach the soil. Also the slightly higher summer air temperatures, the greater air movement through the stand, and the thinner layer of litter would tend to produce a greater range of temperature. Differences between plots decreased rapidly with increasing depth.

The Rotomahana "mud", which forms the topsoil to a depth of about 6 in. over the area in which the plots were situated, is of only local occurrence and is not typical of the topsoil found over the large areas of pumice lands that have been afforested. As can be seen from Fig. 2, a relatively small amount of added moisture is needed to lower its moisture tension from wilting point to field capacity. When dry it is hard and compact; when wet, sticky and relatively impervious. At all times it tends to act as a "sealing" layer over the coarser and looser pumice layers beneath. These points should be borne in mind when considering the results reported in this paper. Also, the mean air temperatures during the summer of 1954–55 were slightly higher than average and the rainfall was about 5 in. below average.

Work which is now being carried out in a more representative pumice soil in Kaingaroa Forest, Rotorua Conservancy, will be reported on later.

Conclusions

Fibreglass soil-moisture units are superior to gypsum units of the type used because of a more rapid response to changes in soil-moisture content at the beginning of a drying cycle.

Even in a summer which is drier than average, the soil under a stand of radiata pine in Whakarewarewa Forest does not dry out appreciably at depths greater than 12 in. The drying out of the surface soil to wilting point has no apparent effect on the trees.

The surface-soil temperature changes are correlated with changes in air temperature, but soil depth and other factors such as density of canopy and air movement have modifying influences. At a depth of 9 ft 9 in., slight temperature changes occur, but these lag behind the major surface-soil changes by several months.

Compared with the more widespread pumice soils, Rotomahana "mud" has a low moisture-holding capacity and has physical properties which make it relatively impervious to water. Therefore current experiments in Kaingaroa Forest may give results differing somewhat from those recorded in this paper but more representative of conditions in the large areas of afforested pumice lands.

Acknowledgements

The author is indebted to W. G. Warren for the statistical work in this paper, and to R. G. Moore and C. J. Kerr for carrying out the

meteorological readings. The help of Mr. J. W. Holmes, CSIRO, who supplied the pF-resistance graph for the gypsum units, is gratefully acknowledged.

REFERENCES

Aitchison, G. D. 1953: Simplified Measurements of Soil Temperature and of Soil Moisture. *Aust. J. appl. Sci. 4:* 418-26.

Aitchison, G. D.; Butler, P. F.; Gurr, C. G. 1951: Techniques Associated with the Use of Gypsum Block Soil Moisture Meters. *Ibid. 2:* 56-75.

Bouyoucos, G. J.; Mick, A. H. 1940: An Electrical Resistance Method for the Continuous Measurement of Soil Moisture Under Field Conditions. *Mich. agric. Exp. Sta. Tech. Bull. 172.*

Colman, E. A. 1950: Manual of Instructions for use of the Fibreglass Soil-Moisture Instrument. *Calif. For. Range Exp. St.*

Colman, E. A.; Hendrix, T. M. 1949: The Fibreglass Electrical Soil Moisture Instrument. *Soil Sci. 67 (6):* 425-38.

Grange, L. I. 1929: A Classification of Soils of Rotorua County. *N.Z. J. Sci. Tech. 11:* 219-28.

————1931: Volcanic Ash Showers. *Ibid. 12:* 228-40.

N.Z. Soil Bureau 1954: General Survey of the Soils of North Island, New Zealand. *N.Z. Soil Bur. Bull. n.s. 5.*

PHOTOSENSITIVITY DISEASES IN NEW ZEALAND

XIV. THE PATHOGENESIS OF FACIAL ECZEMA

By D. McFarlane[1], J. V. Evans[2], and C. S. W. Reid[3], Animal Research Station, Department of Agriculture, Wallaceville

(*Received for publication, 5 August 1958*)

Summary

The essential pathology of facial eczema in the sheep is an acute cholangitis which in severe, acute, or chronic cases proceeds to obliteration of the duct by fibrous tissue. This latter phenomenon is immediately followed by ductule hyperplasia, canalicular infarction and hepatocellular necrosis and later by cicatrisation of those areas drained by obliterated bile ducts and compensatory hyperplasia of the non-affected portions of the liver.

Introduction

Cunningham *et al.* (1942) first described in detail the clinical, pathological, and biochemical characteristics of facial eczema. Since then a series of papers by Wallaceville and Ruakura workers have given the results of the work carried out on this problem. These include two papers by Evans *et al.* (1957) and Perrin (1957), in which is described the pathology of the guinea-pig liver associated with the feeding of dried grass and extracts from such grass collected during periods when the pasture caused facial eczema in sheep. In the original paper by Cunningham *et al.* (1942), Hopkirk described the essential lesions of facial eczema as being progressive fibrous thickening of the walls of the bile ducts which in some instances led to complete obliteration of the lumen, often associated with damage to the lining epithelium. An associated and dramatic histological change was a marked increase in bile capillary tissue with loss of liver parenchyma. A grossly distorted liver was a common end result. In the above paper the emphasis was placed on the morbid anatomical and histological lesions rather than on pathogenesis of those lesions and one only macrophotograph of a grossly distorted liver was published.

In this paper emphasis is placed on the pathogenesis of facial eczema and a series of photographs illustrating the essential lesions is presented.

Material and Methods

Liver sections stained with haemalum and eosin and their parent paraffin blocks were available from naturally occurring outbreaks of facial eczema, and from sheep fed dried toxic grass for the years 1938 to 1947. From 1947 to 1952 the morbid anatomy and histology was studied in naturally occurring outbreaks. The incidence and severity of the

Present addresses:

1. The Gisborne Veterinary Club, Gladstone Road, Gisborne, New Zealand.
2. The University of New England, Armidale 5N, N.S.W., Australia.
3. Plant Chemistry Laboratory, Dept. of Scientific and Industrial Research, Palmerston North, New Zealand.

disease within a flock was assessed by distributing the livers of lambs from single farms in the following five classes, i.e. those livers showing:

1. No visible lesions (Normal).
2. Focal lesions affecting less than about 5% of liver parenchyma (Focal).
3. Lesions affecting about 25% of the parenchyma (Mild).
4. Approximately 50% affected parenchyma (Moderate).
5. More than 75% of liver tissue affected (Severe).

All livers subsequently used for histology were first divided into right and left halves at a point close to the functional separation of the right and left lobes. Each half of the liver was then cut into slices (1 cm thick) transverse to the direction in which the main bile ducts run. The slices were fixed flat in 4% formaldehyde for four days in which three changes of the formaldehyde were made. Representative blocks were taken from the slices and the unused tissue stored in quart jars in case it became necessary to follow the developing lesions in either direction. Because of the method of cutting the liver, it was easy to reassemble the slices and so obtain the contiguous tissue to any identified paraffin block. Most of the blocks were embedded in paraffin and stained in the following ways. Haemalum and eosin, Weigert's elastica stain, Mallory's phosphotungstic acid haematoxylin, Gordon and Sweet's reticulin, and Picro-Mallory.

Blocks were also cut by frozen-section methods and stained for fat using Sudan 3 and 4.

Results

Histopathology

The essential lesion in facial eczema is a cholangitis affecting all but the very small lobular ductules. There is a wide range in the nature of

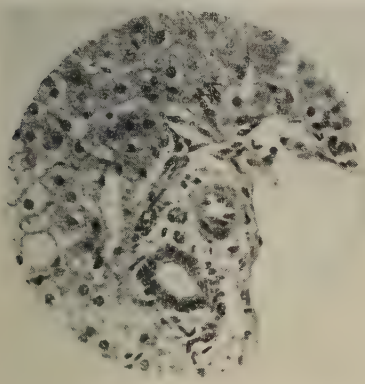


Fig. 1

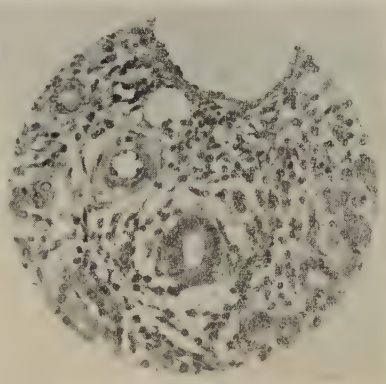


Fig. 2

Fig. 1: H. & E. × 270—Towards 6 o'clock a bile duct shows very early mild periductal oedema. The epithelium shows no gross abnormalities.

Fig. 2: H & E × 200—Towards 6 o'clock a bile duct shows obvious periductal oedema. The epithelium shows no gross abnormalities.

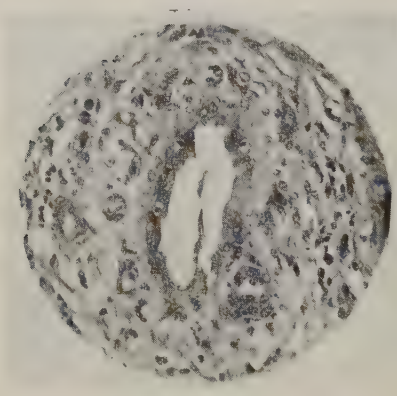


Fig. 3

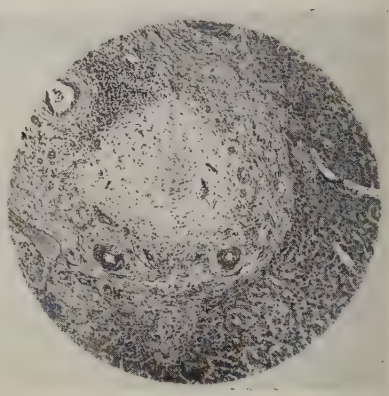


Fig. 4

Fig. 3: H & E × 200—A bile duct showing epithelial degeneration and necrosis with periductal oedema and early fibroblastic reaction.

Fig. 4: H & E × 50—The central paler circular zone is a mass of granulation tissue replacing a bile duct. At 10 o'clock there is a bile duct with periductal fibrosis.

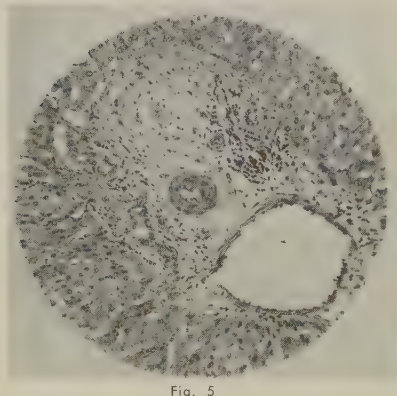


Fig. 5

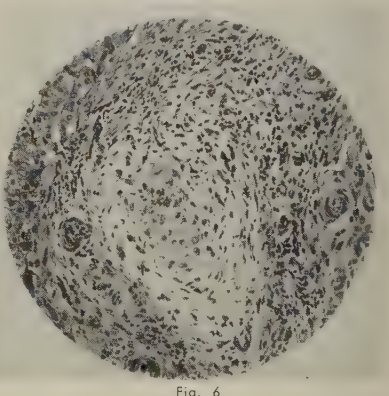


Fig. 6

Fig. 5 & 6: H & E × 100—A small (5) and a larger (6) bile duct showing occlusion and fibrous replacement.

the cholangitis depending on the concentration of the toxin and the length of time for which it is present in the bile. Thus in some naturally occurring cases extreme lesions have developed within seven days of ingesting highly toxic pasture and in other cases relatively mild lesions after six weeks of ingesting almost continuously mildly toxic grass.

It is also important to realise that it is very rare for all ducts to be affected and that affected ducts are all in different stages of development of the disease. *In severe acute cholangitis* there is widespread necrosis (Fig. 3) of the duct epithelium and with this in the early stages is associated a severe periductal oedema. The stage of oedema is

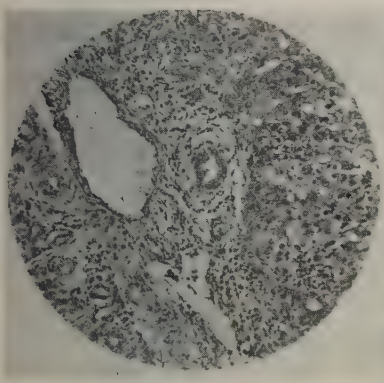


Fig. 7

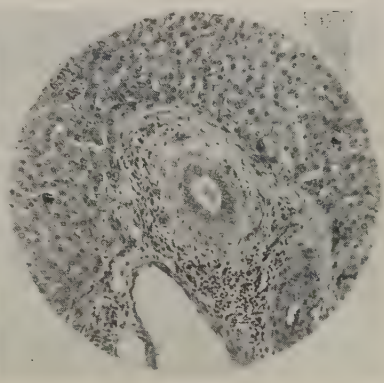


Fig. 8

Fig. 7: H & E × 100—In the centre a duct shows obvious periductal oedema and degenerative and catantal epithelial changes (cf. Fig. 1 & 2).

Fig. 8: H & E × 100—The duct in the centre shows disinct periductal fibrosis and minimal epithelial changes.

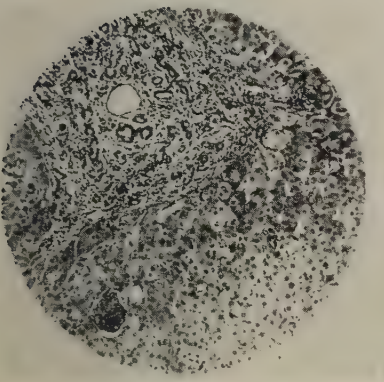


Fig. 9

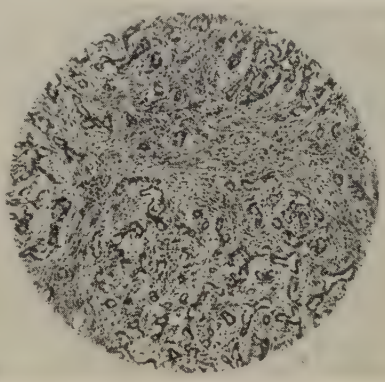


Fig. 10

Fig. 9: H & E × 85—The vein towards 11 o'clock is surrounded by hyperplastic ductules encroaching on the liver parenchyma on the left.

Fig. 10: H & E × 85—This is the final stage when that part of the liver parenchyma drained by an occluded bile duct has disappeared and has been replaced by a small volume of fibrous tissue rich in proliferated bile ductules.

followed by a distinct hypercellularity due to the emigration of haemal leucocytes and to the proliferation of fibroblastic cells. These latter proliferate rapidly, forming typical granulation tissue which frequently replaces lengths of the duct for over 3 cm (Fig. 4, 5, 6).

In the later stages, the granulation tissue undergoes maturation and may finish up as an insignificant cord of dense fibrous tissue whose

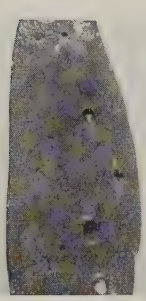


Fig. 11

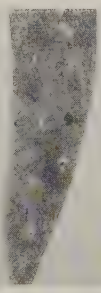


Fig. 12

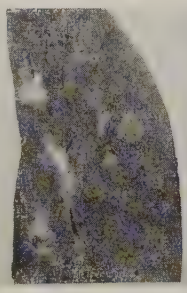


Fig. 13

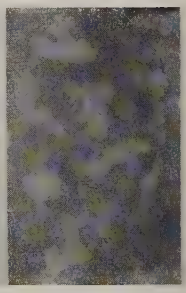


Fig. 14

Fig. 11: Unfixed × ½—The four large holes (three dark, one light) are sublobular veins. The majority of the smaller stellate holes are portal veins in normal portal tracts. Neither artery nor bile duct can be seen in normal portal tracts of this size.

Fig. 12: Unfixed × ½—A section of approximately the same level as in Fig. 11. The small stellate portal tracts clearly show the white (periductal) fibrosis. The pale strands surrounding the portal tracts are composed of hyperplastic bile ductules.

Fig. 13: Unfixed × ½—Three large and two smaller portal tracts showing massive periductal fibrosis.

Fig. 14: Unfixed × ½—The diaphragmatic surface of the left lobe of the liver shows numerous light variegated areas of affected liver. These are of different ages, consist mainly of proliferated bile ductules, and can be traced back to occluded bile ducts.

presence is frequently indicated only by portal tracts in which there is no bile duct present.

In the milder and more chronic type of outbreak, the duct epithelium shows changes varying from slight alterations in cell height and staining characteristics to the typical phenomena of acute catarrh (Fig. 7). The periductal tissues may show nil to moderate oedema and cellularity (Fig. 1, 2) while the fibroblastic changes are slower and in some cases ducts may show progressive fibrosis with little of the more active granulation tissue reaction (Fig. 8). Depending on the concentration of toxin and the time over which it acts, the lesions described may progress to complete obliteration of lengths of ducts or may cease at any stage short of this.

Sequelae to Duct Obliteration

Rapid obliterative cholangitis is frequently followed by the accumulation in the larger bile ducts of clear mucus and/or inspissated bile which may lead to fusiform dilations with a diameter of 5 cm in the larger bile ducts. Within some lobules, rapid occlusion of ducts leads to an accumulation of bile in the intercellular canaliculi which when excessive is associated with focal lobular necrosis. The most dramatic sequela of duct obliteration, however, is the overgrowth of bile ductules around the margins of the lobules drained by the affected duct (Figs. 9, 10). This overgrowth is so rapid in the early stages as to resemble neoplasia. Ultimately the proliferating duct epithelium replaces the parenchymal cells. This phase is followed by progressive fibrosis of the affected tissue, the final appearance of which is an area of mature fibrous tissue with cords of ductule epithelium scattered through

it (Fig. 10). The portions of the liver drained by patent ducts undergo compensatory hyperplasia leading to the development of liver tissues in which the normal portal tracts are distributed irregularly in parenchyma in which lobular structure can no longer be recognised.

Morbid Anatomy

The first macroscopic lesion that can be seen is a distinct oedematous or fibrous thickening of bile ducts whose diameter is from 1–3 mm (Fig. 12, 13). These are usually best seen 2–3 cm in from the margins of the left or right lobes by making there an incision transverse to the direction of the distribution of ducts. In this area normal bile ducts can just be seen as holes with no discernible wall (Fig. 11). As the cholangitis progresses to complete obliteration, the cone-shaped area of liver drained by the occluded duct develops irregular strands of pale oedematous or fibrous tissue spreading in an arborescent fashion (Fig. 10, 12). At this stage, affected subcapsular areas may protrude slightly above the surface of the normal surrounding liver, and have a slightly granular surface (Fig. 14). As the sequela of fibrosis comes to dominate, the affected areas undergo typical cicatrisation.

The above duct lesions and their sequelae are the basic pathology of facial eczema. The wide range of morbid anatomical features met with depends, as already stated, on the concentration and duration of action of the toxin and makes systematic descriptions difficult. The morbid anatomical features are best described in two stages, *early* and *late*.

Early Stages. In the 1938 outbreak, the main morbid anatomical features were very rapid duct occlusion with distention of larger ducts with clear mucus and/or inspissated bile.

In subsequent years this feature has been seen only occasionally, and in most outbreaks the diseased livers have been of two main types. In the first type the affected ducts reach the occluded stage at a wide variety of times so that affected livers have a variegated appearance due to the pale cones of liver affected with facial eczema lesions of varying ages abutting on Glisson's capsule (Fig. 12). The diameter of the bases of those irregular cones are from 1–5 cm or more, depending on whether or not confluence of lesions has taken place. The intervening liver tissue is normal in appearance.

If such a liver is sliced in the way described it is easy to find the occluded ducts leading to the altered areas and, in addition, non-occlusive fibrosis of the larger ducts is usually quite obvious.

In the second type, nearly all ducts appear to become affected to much the same extent and complete occlusion seems to affect smaller ducts. The unopened liver shows no variegation although it is enlarged, olive green in colour, and definitely firmer than normal. On routine slicing, the periductal fibrosis is more difficult to see. If the friability of the liver is regularly tested by squeezing slices between the thumb and forefinger, the dense fibrotic texture of this type of facial eczema is readily distinguished from the normal soft slices.

Late Stages. The end result in the gross anatomy of affected livers depends on the distribution of occluded ducts, since the areas drained by these cicatrise while the remaining functional areas undergo hyperplasia. Thus fibrotic scars may be few and small and almost

unnoticeable, or they may affect so much of the liver that it is grossly shrunken and/or distorted in shape.

Discussion

The two most interesting features of facial eczema are the extreme variability in the nature, severity, and distribution of lesions in animals grazing as one flock and the nature of the essential lesion. The former could hypothetically be ascribed to the variations in grazing behaviours of animals in the same paddock or to large differences in susceptibility between animals. Considering the nature of the pathology a liver disease due to the ingestion of a substance which, on entering the portal circulation and thence to the bile gives rise to a focal or diffuse cholangitis ultimately leading to fibrous replacement of variable lengths of the bile duct is very unusual. The main sequelae to this lesion, i.e. canalicular infarction, focal necrosis, ductule hyperplasia, distention of ducts with mucus and/or bile, have all been duplicated by experimental ligation of the bile duct in rats by Cameron (1935) and Cameron and Hasan (1958), and in sheep by Quinn (1933) and by Swan, Reid, Evans, and McFarlane (unpublished).

It is idle to conjecture on the nature of the toxin, although there are several interesting features worthy of comment. Thus the toxin appears to cause no gross damage to abomasal or intestinal mucosa. The parenchyma of the liver and the perilobular ductule epithelium show no distinctive lesions using the histological methods described. The smallest size of duct affected appears to be fairly constant as judged firstly by measurement, i.e. about 200 microns, and secondly by the size of the affected dependent liver lesions. There appears to be no upper limit in size of ducts capable of being affected. Such features could be related to the known occurrence of bile concentration, but so far no attempts appear to have been made to relate the degree of biliary concentration to the duct size. The marked variability of localisation of lesion with a distinct favouring of the left lobe might be explained by portal streaming effects. Preliminary investigations of this phenomenon in the sheep by Reid, Evans, and McFarlane (unpublished) brought to light many problems in anaesthesia and surgical method. In particular undue excitement prior to or during anaesthesia led to extremely variable results.

References

Cameron, G. R. 1935: *J. Path. Bact. 41:* 283-8.
Cameron, G. R.; Hasan, S. Muzassar. 1958: *Ibid. 75:* 333.
Cunningham, I. J.; Hopkirk, C. S. M.; Filmer, J. F. 1942: *N.Z. J. Sci. Tech. A24:* 185-92.
Evans, J. V.; McFarlane, D.; Reid, C. S. W.; Perrin, D. D. 1957: *N.Z. J. Sci. Tech. A38:* 491-6.
Perrin, D. D. 1957: *Ibid. A38:* 669-79.
Quin, J. I. 1933: *Onderstepoort, J. vet. Sci. 1:* 505-26.

CORRIGENDA

Volume 1

page 897, the 4th line should read:
fruit compared with 1.5% to 7.5% for the other varieties. Recent

page 969, 6th para., 1st line, for Nitrate *read* Nitrite

NEW ZEALAND JOURNAL OF SCIENCE

Volume 1, No. 4, December 1958

CONTENTS

Racial Differences in Size and Growth in the New Zealand Snapper. *Alan R. Longhurst.*

Biological Evaluation of Organic Pollution of New Zealand Streams. *Allan Hirsch.*

A Reconnaissance Survey of Pollution in the Manawatu River. *J. K. Johannesson.*

The Occurrence of an Argasid Tick in New Zealand. *L. J. Dumbleton.*

Alate Aphids Trapped in Auckland, New Zealand, using Moericke Colour Traps. *K. P. Lamb.*

Foods of the Australian Opossum (*Trichosurus vulpecula* Kerr) in New Zealand Indigenous Forest in the Orongorongo Valley, Wellington. *R. Mason.*

Note on the Efficiency of Aphid Trapping. *H. R. Thompson.*

Contributions to a Chromosome Atlas of the New Zealand Flora—I. *J. B. Hair* and *E. J. Beuzenberg.*

Index to Volume 1.

CONTENTS

	PAGE
The Insect Complex of Sown Pastures in the North Island. II. The Hemiptera as Revealed by Summer Sweep-sampling. *R. A. Cumber*	1
Effects of Ingestion of Paraffins by Ruminants: V. Blood Plasma Prothrombin Times of Cows Drenched with Heavy Liquid Paraffin. *C. S. W. Reid* and *H. E. Hutchings*	26
VI. Ingestion of Heavy Liquid Paraffin by Cows near Parturition in Relation to Milk Yield and Composition and to Fat-soluble Vitamin Content of the Butterfat during the Early Stages of Lactation. *W. A. McGillivray, F. H. McDowall,* and *C. S. W. Reid*	35
Bloat in Cattle: XI. The Foaming Properties of Proteins, Saponins, and Rumen Liquor. *J. L. Mangan*	47
XII. The Effect of Orally Administered Penicillin on Body Weight of Lactating Cows, on Milk Production, and on Production and Composition of Fat. *A. T. Johns, F. H. McDowall,* and *W. A. McGillivray*	62
A Method of Estimating the Botanical Composition of the Diet of Sheep. *Barbara H. Croker*	72
Cercospora Leaf Spot on Rhododendron (*Cercospora handelii* Bubak). *G. W. F. Maris McArthur*	86
Rotomahana "Mud" Not Cobalt Deficient. *I. J. Cunningham, C. R. Taylor,* and *A. G. W. Stevens*	90
Nitrate Reduction in the Rumen of the Grazing Sheep. *N. D. Jamieson*	96
Intraspecific Competition as a Factor in Ryegrass Breeding. *S. O. Fejer*	107
Biological Control Factors Influencing Populations of Oak Leaf-miner, *Lithocolletis messaniella* Zeller, in New Zealand including the Introduction of Parasites. *B. B. Given*	124
High Molybdenum Intake and the Thrift of Young Sheep. *I. J. Cunningham* and *K. J. Hogan*	134
The Effect of Sulphate and Molybdenum on Copper Metabolism in Cattle. *I. J. Cunningham, K. G. Hogan,* and *B. M. Lawson*	145
The Effects of Topdressing and Oversowing Hill Pastures. *F. E. T. Suckling*	153
Progressive Reduction of Storage Temperature for Apples. *C. S. Padfield*	165
Insects and Mites Associated with Ryegrass. *D. C. M. Manson*	169
Two Diseases of Plum Causing Distortion and Internal Necrosis of Fruits. *E. E. Chamberlain, J. D. Atkinson,* and *J. A. Hunter*	174
Soil-moisture and Soil-temperature Studies under Radiata Pine, Whakarewarewa Forest, 1954–55. *G. M. Will*	184
Photosensitivity Diseases in New Zealand. XIV. The Pathogenesis of Facial Eczema. *D. McFarlane, J. V. Evans,* and *C. S. W. Reid*	194

SUBSCRIPTIONS. Subscriptions will be accepted only on advance payment and only for a full calendar year. Price: 30s. per annum; single copy 5s.; post free (surface mail). Published in alternate months, starting February. Subscriptions should be forwarded to the Publications Officer, Department of Scientific and Industrial Research, P.O. Box 8018, Wellington, New Zealand. Cheques should be made payable to the Department of Scientific and Industrial Research, and if drawn on banks outside Wellington, must have exchange added. Subscribers are requested to give their name and full postal address.

This journal may also be ordered through The High Commissioner for New Zealand, 415 Strand, London W.C.2, or through any bookseller.

Printed by Coulls Somerville Wilkie Ltd.,
Wellington, New Zealand.